WATER USE AND THE NUTRITIONAL WATER PRODUCTIVITY OF BUSH TEA (ATHRIXIA PHYLICOIDES DC.)

by

Muneiwa Rumani

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Bush tea (*Athrixia phylicoides* DC.)

PREFACE

The study was conducted by an MSc student in the Department of Horticulture at the School of Agricultural, Earth and Environmental Sciences in the College of Agriculture, Engineering and Science at the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The Water Research Commission (WRC) of South Africa provided financial assistance for the study under WRC Project No. 2020-2021/00420, entitled "**The Water Use and the Nutritional Water Productivity of Bush Tea**". The study aimed to investigate the water use and nutritional water productivity of bush tea. The contents of this work have not been submitted to another university, and the results presented are the outcome of the candidate's own research.

[redacted]

Signed: Professor Fhatuwani Nixwell Mudau

(Main Supervisor)

Date: 16 November 2023

Signed: Professor Tafadzwanashe Mabhaudhi

(Co-Supervisor)

Date: 16 November 2023

DECLARATION 1: PLAGIARISM

I, **Muneiwa Rumani**, declare that:

(i) the research reported in this dissertation, unless specifically acknowledged as being sourced from other papers, is my original work;

(ii) this dissertation has not been submitted in full, or in part, for any degree or examination to any other university;

(iii) unless identified as being sourced from other people, this dissertation does not contain any data, photos, graphs or other information that belongs to another person;

(iv) unless noted as being sourced from other studies, this dissertation does not contain any writing from other people. Where other written sources are cited, then:

  a) their general information has been referenced, but their precise words have been rewritten; and

  b) their writing has been placed inside quotation marks when it contains their exact words.

(v) I have specifically stated my involvement in the work whenever I have used material that was later published.

(vi) This dissertation is mostly a compilation of the content that I have written and published in journals or given as oral and poster presentations at conferences. In certain instances, extra content has been added.

[redacted]

Signed: **Muneiwa Rumani**

Date: 16 November 2023

DECLARATION 2: PRESENTATIONS

Chapter 3

Muneiwa Rumani[1*], Tafadzwanashe Mabhaudhi[2,3], Anza-Tshilidzi Ramabulana[4], Maanea Lonia Ramphinwa [5], Ntakadzeni Edwin Madala[4], Fhatuwani Nixwell Mudau[1*]. Response of Physiological parameters, Nutritional Water Productivity, and Bioactive compounds to Varying Water Regimes of Bush tea (*Athrixia phylicoides* DC.). **Postgraduate Research & Innovation Symposium (PRIS) Conference.**

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Signed: **Muneiwa Rumani**

Date: 16 November 2023

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DEDICATION

I would like to honor my mother, Mrs. Tshifularo Margret Rumani, by dedicating this master's degree to her:

Ndi kho livhuwesa u tikiwa nga thabelo dzavho mma!

LIST OF ABBREVIATIONS

A/C_i :	CO_2 assimilation rate/inter-cellular CO_2 concentration
A :	Net CO_2 assimilation rate
AES :	Alternative Electron Sinks
ANOVA:	Analysis of Variance
ARC :	Agricultural Research Council
AWS :	Automatic Weather Station
BPI :	Base Peak Intensity
C_i/C_a :	Ratio of inter-cellular and atmospheric CO_2
Ca :	Calcium
CERU :	Controlled Environment Research Unit
CGA :	Chlorogenic Acid
CHS :	Chalcone Synthase
C_i :	Inter-cellular CO_2 concentration
CRBD :	Complete Randomised Block Design
CV :	Coefficient of Variation
CWR :	Crop Water Requirements
d.f :	Degrees of freedom
ESI :	Electrospray ionization
ET_a :	Crop Water Requirement
ET_o :	Average Reference Evapotranspiration
ETR/A :	Relative measure of electron transport to oxygen molecules
ETR :	Electron Transport Rate
FAO :	Food and Agriculture Organisation

Fe : Iron

FNS : Flavone synthase

F_v/F_m : Maximum quantum efficiency of photosystem II photochemistry

GNPS : Global Natural Products Social Molecular Networking

g_s : Stomatal conductance

ha : hectares

HOBO : Honest Observer By Onset

ICFR : Institute for Commercial Forestry Research

IRGA : Infrared Gas Analyzer

K : Potassium

K_c : Crop Coefficient

Kg : Kilograms

KZN : KwaZulu-Natal

L : litre

LAN : Limestone Ammonium Nitrate

LC : Liquid Chromatography

LCF : Leaf Chamber Fluorometer

Lhr^{-1} : Litres per hour

LSD : Least Significant Difference

Mg : Magnesium

Mn : Manganese

MN : Molecular Network

MS : Mass Spectrometry

MSI : Metabolomics Standard Initiative

mm : millimetres

N : Nitrogen

NAP : Network Annotation Propagation

NC : Nutritional Content

Ns : Non-significant

NUCS : Neglected and Under-utilised Crop Species

NWP : Nutritional Water Productivity

NY : Nutritional Yield

P : Photosynthesis

P : Phosphorus

PAL : Phenylalanine ammonia-lyase

PAR : Photosynthesis Active Radiation

QC : Quality Control

qN : Non-photochemical quenching

qP : Photochemical quenching

QTOF : Quadruple Time-of-Flight Tandem Mass Spectrometry

RH : Relative Humidity

SA : South Africa

RSA : Republic of South Africa

SC : Stomatal Conductance

SDG : Sustainable Development Goal

SSA : Sub-Saharan Africa

SWC : Soil Water Content

T : Transpiration rate

UKZN : University of KwaZulu-Natal

USA : United States of America

WAP : Weeks After Planting

WP : Water Productivity

WUE : Water Use Efficiency

WUE_i : Intrinsic Water Use Efficiency

WUE_{ins}: Instantaneous Water Use efficiency

Zn : Zinc

Φ_{PSII} : Effective quantum efficiency of PSII photochemistry

ABSTRACT

Bush tea (*Athrixia phylicoides* DC.), is a naturally growing South African herb. It is highly valued for its medicinal attributes and the potential of herbal tea industrialization could help to mitigate water scarcity challenges. Nevertheless, comprehensive investigations into the water utilization, nutritional water productivity, and cultivation of bush tea remain unexplored. Hence, the study's primary objective was to assess how bush tea elucidates its development, how much water it uses and the quality attributes when cultivated in contrasting environments. Studies on water utilization, Nutritional Water Productivity (NWP) and of bush tea have not yet been explored. The study was undertaken during the year 2022 and 2023 growing seasons. A Complete Randomised Block Design (CRBD) was used, consisting of three water regimes, viz. 100%, 30% and the control (stress and rainfed) of the crop water requirements, replicated three times. The growth, development and productivity were measured weekly at budding stage, while Water Productivity (WP) and yield assessments were carried out at harvest. Thereafter, the concentration of micro and macronutrients was then analysed, and bush tea leaves were freeze-dried to determine the biochemical analysis. The results derived from Water Productivity (WP) and Nutrient Content (NC) measurements were used to determine the Nutritional Water Productivity (NWP). Under a controlled environment, the findings indicated that the 100% ET_a water treatment yielded a higher crop output (95.62 $kg.ha^{-1}$) compared to the 30% ET_a water treatment (60.61 $kg.ha^{-1}$) and the control (12.12 $kg.ha^{-1}$). Similarly, WP was more favorable under 100% water treatment in comparison to the 30% water treatment. Based on the mean values, the highest ($NWP\_{_{Ca,\ Cu,\ Fe,\ Mn}}$ &$_{Zn}$) was attained under a 30% ET_a water treatment which was significantly higher than the 100% ET_a water treatment and stress (control) of the crop water requirements. Similar to the controlled environment experiment, the 100% ET_a water treatment yielded more crop output (259.1 Kg. ha^{-1}) under field conditions, compared to the 30% ET_a water treatment (171.2 kg. ha^{-1}) and control (stress) (68.2 kg. ha^{-1}). The bush tea leaf extracts were shown to be phytochemically rich, with a variety of physiologically active metabolites that were distributed differently within each water application. In conclusion, bush tea thrives well under limited water application and stress conditions, and that its yield is satisfactory, without compromising its nutrients across the varying water regimes. Consequently, NWP in bush tea cultivation serves as a valuable indicator of its potential contribution to nutritious food in water shortage areas. Additionally, the study's findings also emphasized different kinds of metabolites, including a variety of terpenoids, chlorogenic acids, lipids, and flavonoids. Under different water levels and constrained areas, this research also decoded newly formed metabolomes of bush tea for the first time. As a result, the study reveals new knowledge that will be valuable to other researchers working on the domestication and cultivation of bush tea. Additionally, it seeks to improve the economic security of rural communities by increasing access, availability, utilization, and stability of bush tea supply. The study primarily concentrated on how the influence of varying water regimes affect bush tea growth productivity, NWP, and the phytochemicals of bush tea plant species.

Keywords: **Herbal tea, growth development, water regime, water use efficiency, phytochemicals, molecular network, yield, nutrient content**

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

1.1 Background

South Africa is a country facing rapid water shortages due to water deficit (Singels *et al*., 2010). The average rainfall in the country is about 500 mm, lower than the average annual rainfall of 860 mm per year (Mabhaudhi, 2014; Crétat *et al*., 2012). In 85% of the country, most of the people lives in rural areas (Shackleton *et al*., 2008). Food insecurity, poverty, unemployment, and rain-fed agriculture are common in these communities (Shackleton *et al*., 2008; Beddington *et al*., 2011; Wenhold *et al*., 2012). Improving agriculture in rural communities is recognized to enhance food security, strengthen families in reducing poverty, and strengthen rural energy (RSA, 2010). It is stated that the existing agricultural activity in these places is excessive and devoid of the innovation necessary to promote rural economic growth (Beddington *et al*., 2011). Facing these problems, agricultural models need to adapt to climate and economic changes and adopt new models that will create new niche markets. One strategy involves multi-purpose medicinal plants having the potential for commercialisation.

Bush tea is a South African herb commonly used by South Africans as an anti-anxiety agent and aphrodisiac (Mabogo, 1990). This plant grows in arid regions where tree cover is known to be limited (Mudau *et al*., 2006). Historically, this plant has been used as a treatment for heart disease, high blood pressure, acne, fever, colds, cuts, migraines, infected wounds, and sore throats (Nchabeleng *et al*., 2012; Mudau *et al*., 2006). It is also considered as a powerful blood purifier due to its high total polyphenol content Roberts (1990), because it contains a lot of total polyphenols (Mudau *et al*., 2007a). Polyphenols are formed by chalcone synthase (CHS), flavonoid synthase (FNS), cinnamic acid 4-hydroxylase (C4H), is regulated by phenylalanine ammonia lyase (PAL) as well as 4-coumaric acid, shikimic acid, and phenyl Continuous transcriptional regulation, which is also the metabolic precursor of the propionate pathway and CoA ligase (4CL) (Kanazawa *et al*., 2012). Bush tea contains polyphenols as well as quercetin, 3-o-desmethyldidiolone, 5,6,7,8,3',4'-hexamethoxyflavone (Mudau *et al*., 2007a), 5-Hydroxy-6,7, 8, 3',4 ',5'-hexamethoxyflavan-3-ol (Mashimbye *et al*., 2006), tannins (Mudau *et al*., 2007b) and antioxidants (Daniel *et al*. 2007). Studies have also shown that tea has anti-depressant and positive effects (Mashimbye *et al*., 2006; Mudau *et al*., 2007a). Wild plants are under great pressure due to demand from local and international markets that require other products of plant products (Mudau *et al*., 2007a). According to Wiersum *et al.* (2006), found that overconsumption of bush tea in many areas resulted from greater energy use, posing a threat to local biodiversity. Another option is large-scale cultivation to ensure economic profitability (Mudau *et al*., 2022). It is important to remember that water is important for growth and quality of the bush tea, and many herbs need to be accounted for as their chemical content and accumulation has been shown to affect its growth (Maedza, 2015; Mudau *et al*., 2007a).

A clear understanding of its water and nutrient requirements will assist farmers to optimise its production in the different growing regions. Furthermore, it influences marketing and determines the affinity of consumers to accept bush tea products. Odav *et al*. (2007) found that South Africa has many medicinal tree and shrub species, the socio-economic value of which, as well as their water productivity and NWP, are underexplored. The quantification of NWP is still in a relatively early stage and has been used in only a few cases. For example: Chibarabada *et al.* (2017), who evaluated the NWP of selected cereal legumes [Bambara groundnut (*Vigna subterranean*), cowpea (*Vigna unguiculata*), groundnut (*Arachishypogaea*) and dry bean (*Phaseolus vulgaris*)]; and Nyathi *et al*. (2019) reported on leafy vegetables [amaranth (*Amaranthus cruentus*), the spider flower (*Cleome gynandra*), sweet beet (*Beta vulgaris*)] and orange-fleshed potato (*Ipomoea batatas*).

According to a study by Mabhaudhi *et al.* (2018), stated that “food crops improve the health and well-being of poor rural households.” As far as we are aware, no research has previously examined the water use and nutritional water productivity (NWP) of therapeutic plants like bush tea. Considering this, the purpose of this study is to assess the water usage, NWP and quality characteristics of bush tea in different water level in different regions. Consequently, this study aims to evaluate the quality and growth of different water levels, NWP of bush tea in different areas. The following goals were set in order to validate the hypothesis and achieve the research objectives, the following specific objectives were set:

- to assess how varying water regimes in contrasting environmental conditions affect the growth, development and productivity, Nutritional Water Productivity (NWP) and yield of bush tea;
- to determine the biochemical/quality analysis of bush tea.

1.2 Hypothesis

Null Hypothesis: The growth development and productivity, nutritional water productivity and quality of the bush tea grown under contrasting environmental conditions will not be affected by varying water regimes.

1.3 Dissertation Structure

The work is in paper form and consists of five chapters, each independent but related to the objectives. Two of these chapters are in manuscript form and information about their proposed publication or current revision status in each journal is duly disclosed in the footnotes on the title page.

Chapter 1 is the current chapter, which is intended to provide a general introduction and outline the problem and specific research objectives.

Chapter 2 is a review designed to discuss the potential of water as an approach to cover the water regimes, and it has used a phytochemical/biochemical analysis to cover the second objective under the quality analysis including the general review of herbal teas. The review examines the comprehensive strategies and mechanisms related to water of various herbal teas, including *Athrixia phylicoides* DC. (Bush tea).

Chapter 3 reports on the first objective. It reports on the growth, development and productivity, yield, nutritional water productivity and bioactive compounds of bush tea under three water regimes namely, control (stress), 30% and 100% water treatments in a controlled environment.

Chapter 4 is similar to the first objective as it involves documenting the water utilisation, NWP, growth development, yield, as well as the phytochemical analysis under three different water regimes (rainfed, 30% and 100% ET_a) of the crop water requirements of bush tea on field condition.

Chapter 5 serves as an extensive general discussion that unifies the separate manuscripts and aligns them with the dissertation's goals. It covers the main conclusions of the dissertation, the difficulties faced during the current research, and suggestions for future studies on bush tea.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

2.1.1 Botany and ecology

Bush tea (*Athrixia phylicoides* DC.) is a plant belonging to the *Asteraceae* family (Mudau *et al*., 2007a). It grows well in the arid country of Southern Africa and has great horticultural potential, as it is valued for its health benefits and nutritional components (Mudau *et al*., 2007a). For decades, rural populations have harvested it as a tea and it has been used traditionally as a medicine, particularly among low-income groups (Mudau, 2006). Throughout history, South Africans first used it as a tea to purify or cleanse the blood and to treat stomach pains, headaches, wounds and cuts. This solution can also be used as a bubble bath (Rakuambo, 2007). Similarly, the foam bath brew can be applied as a lotion onto skin eruptions or cuts (Rakuambo, 2007). The tea is also used to treat coughs and colds, gargle, and treat throat infections and voice loss (Rakuambo, 2007). According to different researchers, the *Athrixia phylicoides* DC. can be commercialised as an herbal beverage with its medicinal properties (Mudau, 2006; Nchabeleng *et al*., 2012; Rakuambo, 2007; Tshikhudo *et al*., 2016; Tshivhandekano *et al*., 2014; van Wyk and Gorelik, 2017). However, nowadays it is used commercially as an herbal beverage (Rakuambo, 2007).

Traditionally, various tribes have used bush tea differently; extracts from soaking the roots and leaves have helped the Venda people to remove or fight parasitic worms, particularly human intestinal helminths (van Wyk and Gorelik, 2017). Furthermore, the Zulu people use the fine twigs and leaves of bush tea as medicinal decorations and herbal teas (Tshivhandekano *et al*., 2013), and they also use the roots to extract essence by boiling them, as a cure for wheezing. South Africa produces rooibos tea, honey-bush tea, and bush tea, which are all indigenous herbal teas (Maudu *et al*., 2010), and they have all been used as therapeutic teas for decades (Mudau, 2006). The quality of herbal teas is one of the most important factors that determine their value in local or international use (Maedza, 2015; Mashimbye *et al*., 2006; Mudau, 2006).

2.2 Botanical aspects

2.2.1 Classification

Bush tea is an erectophile plant named after the genus name *Athrixia*, belonging to the *Asteraceae* (daisy) family, inucleae and *Anthrixiinae* subtrite (Windvogel, 2019). According to Lehlohonolo *et al*. (2013), the specific epithet *phylicoides* relates to its similarity to Rhamnaceae. The genus name *Athrixia* refers to the leaves and is derived from the Greek word *thrix*, meaning "hair". Traditionally, bush tea has many names according to different groups; Bushman stream, Icholocholo, Itshelo, Umtshanelo (Zulu), Mohlahlaishi (Pedi), Sephomolo (Sotho), Mutshatshaila (Venda), bush tea, Bushman's tea, Zulu tea (English), Boesmans tee (Afrikaans) and Luphephetse (Swati) (Mudau, 2006).

Figure 1: Physical appearance of the bush tea (*Athrixia phylicoides* DC.) plant (A), stem and leaves (B) flowers (Tshivhandekano et al., 2014)

2.2.2 Origin and distribution

The researchers found that *Athrixia phylicoides* is widespread from the Soutpansberg mountains in Limpopo as far east as Queenstown, King Williams Town and East London, and throughout KwaZulu-Natal from the coast to the Drakensberg mountains (Joubert *et al*., 2008; Lehlohonolo *et al*., 2013; Marasha *et al*., 2013; Windvogel, 2019). However, A. *phylicoides* is well adapted to different altitudes, from 600 to 2000 m above sea level (Williams, 2013). There are 14 species, the most (nine) of which are found in Africa and Madagascar, as well as in Southern Africa (Mudau, 2006). *Athrixia Angushssina*, *A. elata, A. ger rardi, A. hererophylla,* and *A. phylicoides* are the most common species in South Africa (Joubert *et al*., 2008).

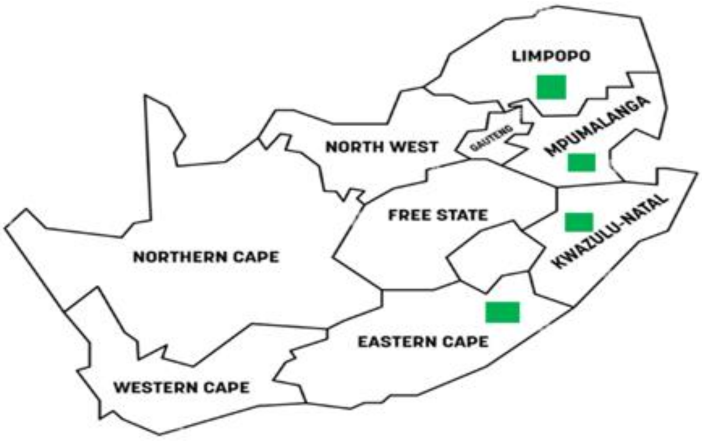

Figure 2: Bush tea (*Athrixia phylicoides* DC.) distribution locations in South Africa

2.2.3 Botanical information

Bush tea (*Athrixia phylicoides* DC.) is an evergreen tree with a white hairy stems, up to 1 m tall (Lerotholi *et al*., 2017). Although the stems appear fluffy and white, bush tea leaves are simple, stem-shaped, alternately linear (30 x 10 mm) to broadly lanceolate, tapering to a sharp point; its part are dark green and shiny below and the upper part is white and the edges are completely or slightly rounded (Tshikhudo *et al*., 2016). In addition, the base of the leaves is usually ear-shaped and has short stems. Shrub teas have stemless or nearly stemless flower heads ending in large sub-corymbose (Mudau *et al*., 2007a). Subsequently, *Camellias* become daisy-shaped with flowers ranging from pink to pink and yellow and bloom every year, depending on the region (Mudau *et al.*, 2007a). Bush tea produces narrow, cylindrical and slender fruits that are 0.01 to 0.06 mm wide. However, the seeds are 4 mm long and have two flowers (Maedza, 2015). Bush tea grows well in the savannahs of South Africa and in densely forested frontier areas, including Limpopo, the Free State, KwaZulu-Natal and parts of the Eastern Cape (Maedza, 2015). Seeds and cuttings are also a good way to propagate them. During summer, mature seeds are usually propagated and harvested (Marasha *et al*., 2013).

2.3 Tea consumption and medicinal use

For many years bush tea is used to cleanse or purify the blood and to treat headaches, infected wounds and cuts (Zaveri, 2006). In Venda culture, the tree is considered a tree that celibates should eat (Gedela *et al.*, 2016), while Sotho and Xhosa people eat it to relieve sore throat and coughs (Mudau *et al*., 2007a). Bush tea extract prepared from brewed roots and leaves is used as an anthelmintic in the Vhembe region of Limpopo Province (Mudau *et al*., 2007b). The tea can also be used to gargle with for throat infections and voice loss, as well as for coughs and colds (Chopade *et al*., 2008). Bush tea is traditionally made by boiling the leaves in water for 10 to 15 minutes before serving it (Windvogel, 2019). Although the younger generation add two to three teaspoons of sugar and fresh milk to bush tea, the elders do not drink it with sugar and milk (Lerotholi *et al*., 2017). In addition, herbalists dry leaves in the shade and give them to diabetics, as well as to purify blood to treat stomach acnes, headaches and bruises (Tshikhudo *et al*., 2016).

2.4 Medicinal importance of herbal teas

Herbal tea are made from the leaves, seeds and or roots of various plants (Zhao *et al*., 2013). For this reason, many herbal teas are used due to their medicinal properties (Zhao *et al*., 2013). Moreover, they have played an essential part in the everyday life of many people throughout history, not only for their taste, but also for their therapeutic efficiency (Nookabkaew *et al*., 2006). Furthermore, coughs, sore throats, fever, pains and headaches were all treated with herbal teas medicinally (Chopade *et al.,* 2008). Conversely, some of them are ingested for their therapeutic benefits, which can aid with relaxation, stomach or digestive disorders, as well as the strengthening of the immune system (Windvogel, 2019). Therefore, black tea, green tea, chamomile, ginger tea, ginseng tea, mint tea and cinnamon tea are therefore some of the most popular herbs (Windvogel, 2019). It has also been that the quality of herbal tea is affected by its chemical composition (Nchabeleng *et al*., 2012). Certain compounds, including polyphenols, flavanols and tannins are found in bush tea leaves (Maedza, 2015). In addition, herbal tea contains various mineral compositions that give herbal tea sensory properties features such as aroma and astringency (Gedela *et al*., 2016).

2.4.1 Medicinal value of herbal tea

People have been using herbal plants as medicine for treating physical disorders for many years (Mudau and Mariga, 2013). According to archaeological research, herbal plants have played a vital role in human bodies and has been used as cosmetics (Marasha *et al*., 2013; Mudau and Mariga; 2013; Saeed *et al.*, 2017; Zhao *et al.*, 2013). Flowers and leaves are also used to enhance flavor, make tea, and make medicine (Islam, 2019). Herbal teas have the best and longest-lasting effects among all medicinal herbs (Qasim *et al*., 2017). In addition, herbal teas have been used to protect human health for a long time (Lee and Lee, 2005).

2.4.2 Phenological stage of herbal tea

The peak growth of the tea plant is the phenological stage of the herbal medicine (Joubert *et al*., 2008). The first leaf in spring, also known as the first flower, is the best leaf (Ebrahimi *et al*., 2008). In addition, when the first leaf is collected, new buds appear in its place, which is called a second flush (Ebrahimi *et al*., 2008). According to several researchers, the more efficient and select process, which involves selecting only the first two leaves and buds after washing, the more carbohydrate reserves are accumulated (Gogoasa *et al*., 2013; Herman *et al*., 2000; Maedza, 2015; Mashimbye *et al*., 2006; Mudau, 2006; Özcan *et al*., 2009). Fresh green tea contains volatile compounds such as polyphenols, flavonols, phenolic acids, amino acids, chlorophyll and other pigments (Nchabeleng *et al*., 2012). Several studies have shown that theaflavin and Rubin contents are highest in the early flowering stage and gradually decrease with season, while the lowest content occurs in the main flowering stage (Lee and Lee, 2005; Lehlohonolo *et al*., 2013; Lerotholi *et al*., 2017; Maudu *et al*., 2012; Tshivhandekano *et al*., 2014).

2.5 Chemical composition of herbal teas

Polyphenols flavanols and tannins are antioxidant compounds used to determine the therapeutic effects of medicinal plants (Wüpper *et al*., 2020). Therefore, properties such as amino acids, carbohydrates, organic acids, vitamins and volatile taste molecules, quality attributes (Tshivhandekano *et al*., 2014). Thus, antioxidants, as the chemical composition of tea, will be discussed.

2.5.1 Antioxidants

Free radicals are formed through the oxidation process and they can cause cell damage by starting chain reactions in the cell (Özcan *et al*., 2009). Antioxidant molecules inhibit other molecules from oxidising, which prevents the chain processes and inhibits further oxidation reactions (Mashimbye *et al.*, 2006). Oxidative stress results from the loss of oxidants or the inhibition of antioxidant enzymes that can damage or kill cells (Özcan *et al*., 2009). Antioxidants are used to treat strokes and neurological illnesses, as many human illnesses, including malignancies, appear to be exacerbated by oxidative stress (Maudu *et al*., 2012). In terms of being a measure for classifying distinct materials, the antioxidant content is used for health benefits (Singels *et al*., 2010).

Antioxidant content refers to substances that protect biological organisms from the harmful effects of severe oxidative processes involving nitrogen and oxygen (Higdon and Frei, 2003). The antioxidant content of tea are attributed to many phenolic substances found or isolated in amphiphilic molecules (Higdon and Frei, 2003). The redox functions of phenolics, which allow them to be reduction agents and oxygen singlets, are primarily responsible for their antioxidant action (Mashimbye *et al*., 2006). Thus, polyphenols, tannins and flavanols are the three most significant compounds found in bush tea leaves (Mashimbye *et al*., 2006).

2.6 Polyphenolic compounds

Polyphenols are a class of compounds produced from organic products that contain many phenolic structural units (Lehlohonolo *et al*., 2013). In addition to hydroxyl groups, heteroatom substitutions and ester bonds are also present in polyphenols (Lehlohonolo *et al*., 2013). Tea polyphenols are natural antioxidants for treating anti-inflammatory drugs (Ebrahimi *et al*., 2008). Polyphenols found in herbal teas have biochemical and physiological properties (Qasim *et al*., 2017). Epigallocatechin-3-gallate is the main polyphenol antioxidant found in green tea (Chopade *et al.*, 2008). In skin cells, epigallocatechin-3-gallate has been shown to reduce the number of free radicals and inflammatory prostaglandins (Chopade *et al.*, 2008). Tea leaves are said to be rich in polyphenolic compounds, accounting for about one-third of the dry mass of dried leaves (Lee and Lee, 2005). According to researchers, these polyphenolic components are responsible for the colour and flavour of the beverage, particularly its stringency (Lee and Lee, 2005; Lerotholi *et al*., 2017; Mashimbye *et al*., 2006).

2.6.1 Plant metabolites

Plant metabolites are small molecules with a low molecular weight, which can be primary or secondary metabolites (Hong *et al*., 2016). Primary metabolites are carbohydrates and amino acids that are responsible for the development of the plant and for stimulating bioactive compounds (Hong *et al*., 2016). Polyphenols, especially lignin, are phenolic components of suberin that show UV/V absorptivity to their aromatic structure and widely used electronic conjugation system, and they also have flowering properties (Semenya and Maroyi, 2019). Polyphenols have a high affinity for proteins and are oxidative with soluble proteins (Özcan *et al*., 2009).

2.6.2 Flavonoids

Flavonoids are a type of secondary metabolites found in plants (Junsi and Siripongvutikorn, 2016). In addition, flavonoids are called vitamins probably because of the affect the permeability of blood capillaries (Junsi and Siripongvutikorn, 2016). Chemical factors, physiological regulators and cell immune responses all play a significant role to secrete plant pigmentation (Wüpper *et al*., 2020). Growth inhibitory activity and pre-uptake activity are influenced by the cyclic adenosine monophosphate (camp) of flavonoid (Zaveri, 2006). In addition to this, flavonoids can reduce the risk of hypertension (Tshivhandekano *et al*., 2014), while also regulating blood lipids by improving the function of endothelial and capillary (Tshivhandekano *et al*., 2014).

2.6.3 Tannins

Tannins are astringent phenolic chemicals that can be found in various plant products (Ebrahimi *et al*., 2008), and they are divided into two groups: hydrolysed and condensed tannins (Ashok and Upadhyaya, 2012). Condensed tannins are polymers containing two to five flavonoid units linked by carbon-carbon bonds that are resistant to hydrolysis (Ashok and Upadhyaya, 2012). Tannins found in herbal tea have been linked to the treatment of cancer and heart disease and reduce the likelihood of platelets clumping together (Wang *et al*., 2019). In general, herbal teas have lower tannin content and higher seasonal variations (Ukoha *et al*., 2011). Tannins can be used for the production of anti-corrosive primers (Ukoha *et al*., 2011). As a result, various researchers have found that herbal teas have less hydrolysable tannins in summer, compared to the autumn, winter and spring seasons (Nchabeleng *et al*., 2012).

2.7 Environmental Conditions

2.7.1 Water stress

Several studies have been conducted to investigate the response of tea (*Camellia sinensis* (L)) to drought stress and soil moisture, as well as water deficit (Ahmed *et al*., 2014; Bhattacharya *et al*., 2014; Shirey and Lamberti, 2010). Four studies (Ahmed *et al*., 2014; Bhattacharya *et al*., 2014; Shirey and Lamberti, 2010; Upadhyaya *et al*., 2011), emphasized that drought causes an increase in phenolic compounds and/or antioxidant activity. In addition, a decrease in the levels of these molecules was detected in two studies, while an increase and decrease in these compounds were detected during drought in two studies (Upadhyaya and Panda, 2004;

Chakraborty *et al*., 2002). Depending on the variety, decreasing soil moisture affects tea bud growth and total polyphenol concentration (Upadhyaya and Panda, 2004). Importantly, although phenolic secondary metabolites initially increased in response to drought, they decreased with drought (Upadhyaya *et al*., 2011).

2.7.2 Light

Among all ecological elements, light is the most important (Tshivandekano *et al*., 2014). It is a source of energy for plants and has been shown to influence photosynthesis. The biosynthesis of phenols and flavonoids requires light, and the production of flavonoids is completely dependent on light (Tshivandekano *et al*., 2014). Five studies investigated the effect of different light conditions such as shading process, light intensity and light quality (Ahmed *et al*., 2014; Bhattacharya *et al*., 2014; Chakraborty *et al*., 2002; Ku *et al*., 2010; Zhang *et al*., 2014). Many studies have shown that shaded green tea has lower levels of the phenolic epigallocatechin and epicatechin compounds and amino acids than unshaded green tea. The content of catechin compounds is high, and there is a good relationship between light and polyphenols (Ku *et al*., 2010; Zhang *et al*., 2014; Zheng *et al*., 2008).

Due to the difference in polyphenol composition, shaded tea has more umami flavor and less astringency than unshaded tea (Ahmed *et al*., 2014; Ku *et al*., 2010; Zheng *et al*., 2008). Researchers have found a relationship between unevenness of shade (10% to 60% shade) and tea quality (Upadhyaya and Panda, 2004; Ku *et al*., 2010; Zhang *et al*., 2014). Three of the lighting experiments found that some terpenoids and other volatiles increased, while others decreased, when the light level changed (Zhang *et al*., 2014). Generally speaking, strong light will cause the amino acid concentration to decrease (Zhang *et al*., 2014). According to Tshivhandekano *et al*. (2013), although approximately 50% diffused sunlight is generally suitable for peak physiological activity of tea plants, it is difficult to provide information on the optimal shade required by tea plants. It has also been shown that *Camellia sinensis* requires prolonged exposure to sunlight with a good day length of 14 hours to achieve good nutrition (Tshivhandekano *et al*., 2013). However, little research has been done on bush tea.

2.7.3 Temperature

Three of four temperature studies found differences between temperature and quality of tea in terms of catechins, phenolic secondary metabolites, and antioxidant compounds/activity, while one study showed that sunlight-induced temperature increased catechin compounds (Lee *et al*., 2010; Tanongkankit *et al*., 2011; Wang *et al*., 2011; Yao *et al*., 2005). According to Yao *et al*. (2005), higher catechin concentrations were associated with lower temperature. The tea's main catechin content, as measured by [(-)-epicatechin gallate, (-)-epicatechin gallate and catechin gallate], is higher in the warmer months and lower in the colder months (Lee *et al*., 2010; Wang *et al*., 2011; Yao *et al*., 2005). Nchabeleng *et al*. (2012, found that changes in the total tannin content of tea plants at different altitudes were negatively related to the minimum and maximum values. The effects of temperature and time on the chemical composition of bush tea were studied Nchabeleng *et al*. (2012), found that temperature affects plant growth due to its importance in regulating physiological and chemical processes in response (Tshivhandekano *et al*., 2013). Temperature therefore has a significant impact on the physical cycle of crops, such as diffusion rate, fluid exchange, product solubility, and the balance and stability of different systems, chemicals, and enzymes (Nchabeleng *et al*., 2012).

2.8 Cultivation Practices

2.8.1 Propagation of bush tea

Plant propagation refers to the process of growing new plants from various sources, such as seeds, cuttings, bulbs and other plants (Marasha *et al*., 2013). The propagation of plants is sometimes called Phyto propagation (Araya, 2007). Cuttings are one of the most common ways of clonal regeneration of broad and narrow green leaves, as well as many garden plants, ornamental shrubs and deciduous tree species (Araya, 2007). This propagation method is said to be one of the most widely used and effective breeding methods (Junsi and Siripongvutikorn, 2016). For this reason, bush tea plant is usually propagated by collecting mature seeds at the end of the summer (Lee *and* Lee, 2005). However, if propagation is done too early or not in time, it may affect the germination capacity and transformation time of fruit seeds (Maedza, 2015). A good tea transplant can be done using top cuttings, and multi-rooted cuttings are still needed for survival. Maudu *et al*. (2011) found that the use of Seradix No 2 hormone top cuts on pine trees led to increased rooting of the top trees. However, the germination requirements (light and temperature) of bush tea seeds are unknown (Araya, 2007).

2.8.2 Weed control

Plants thrive in peatlands because they compete with crops for food, water, light and space Das *et al*. (2017), and are unsuitable for growing crops because they host bacteria, insects and nematodes (Das *et al*., 2017). The best weed control strategies include soil preparation and preemergence and post-emergence herbicide applications (Walia *et al.*, 2011). Additionally, weeds can negatively impact crops by reducing yield and quality (Walia *et al.*, 2011). It is important to defoliate the plant in the early stages of growth, because its three-lobed leaves are used for medicinal products and roots (Walia *et al.*, 2011). Additionally, herbal tea contamination originates from various plants (Liu *et al.*, 2020).

Therefore, weed control is important in the early stages of growth of medicinal plants, including the starch and growth stages (Liu *et al.*, 2020). According to Ediriweera, (2010); Habs *et al*., (2017); Rhoda *et al.*, (2020), plants need to be removed before planting. A broad-spectrum glyphosate herbicide such as Roundup should be applied prior to planting to eliminate as much weed as possible (Rhoda *et al.*, 2020). Additionally, the planter can also be used to kill weeds and other pheasant plants as they establish (Rhoda *et al.*, 2020).

Bush tea compete with weeds for nutrients, moisture, and light (Coleman *et al.*, 2020). According to studies Coleman *et al*. (2020); Das *et al*. (2017); Walia *et al*. (2011), found that uncontrolled plant growth removes half of the nitrogen, i.e., phosphate and potassium balance, left in the tea at its most productive stage. When slow-working pressure is high during crop competition, including light competition, yield loss can be as high as 97% (Ediriweera, 2010). According to experts, antibiotics help control plant growth when used before planting (Walia *et al.*, 2011). Post-emergence herbicides can be used to suppress the spread of unwanted plants between the crop rows (inter-row space) in an established tea-tree crop (Banerjee *et al.*, 2006; Ediriweera, 2010). Topical spraying of some antibiotics after hot weather can be used to control certain diseases (Liu *et al*., 2020). If crop replacement herbicides are scarce, non-selective herbicides can be sprayed on the crop after harvest and before regrowth (Habs *et al*., 2017).

2.8.3 Pest and disease control

Losses due to pests and diseases constitute approximately 10-15% of total production (Banerjee *et al*., 2006). Although the use of chemical pesticides in the last 40 years has not increased the benefits, the development of pesticide resistance, the accumulation of residues and environmental damage (Habs *et al*., 2017). Pesticide use should be brought under control by using Integrated Pest Management (IPM) system, with pesticides only being used when the pest population exceeds the tolerable economic threshold (Liu *et al*., 2020).

According to a review by Ediriweera (2010), the money beetle and damping off are two of the pests and illnesses found in the honeybush, with the sweet-smelling blossoms at the tips of the branches attracting the money bugs (Liu *et al*., 2020). Moreover, they are in charge of pollination (Walia *et al*., 2011). The brown seeds grow in little pods that turn brown as they mature (Liu *et al*., 2020), and as the seed ripens, the pods dry out and break open within a few weeks (Ediriweera, 2010). A fungicide should be used to prevent damping off (Ediriweera, 2010). However, the availability of organic pest and insect control products is restricted, which may influence the decision to remain purely organic (Walia *et al*., 2011).

2.8.4 Harvesting and trading of bush tea

Bush tea is generally harvested during the flowering season, from early October to mid-winter (Maedza, 2015). This is thought to increase the sweetness of the flowers and is therefore necessary compared to harvesting when the leaves are drying in the shade (Mudau *et al*., 2007a). The cutting length promotes resprouting for additional harvest and prevents post-harvest mortality (Lee and Lee, 2005). Since shrubs harvested earlier have softer stems, they provide the best service for the business in the next season and also promote better growth (Lee and Lee, 2005).

Shrub tea is collected and harvested seasonally from the wild (van Wyk, 2011). Bundles of tea plants are carefully knotted together to form broomsticks, and when the plants are not collected for brewing, they are sold through small outlets in stores in Limpopo, Mpumalanga and KwaZulu-Natal (van Wyk, 2011). Like other crops, tea production is determined by many factors, including its genotype, soil, and cultivation techniques used (Semenya and Maroyi, 2019). In addition to planting material, pruning and harvesting patterns, fertilization with macronutrients and micronutrients is one of the main factors to produce significant results and quality products (Mudau *et al*., 2007a).

2.9 Nutrition and Health

2.9.1 Mineral nutrition for plant production

If bush tea is produced on a large scale, it is important to ensure the product is available and consistent quality is maintained (Maudu *et al*., 2012). However, commercialization of the tea plant is unlikely (Maudu *et al*., 2012). In agriculture, crop nutrition is a significant aspect and high-quality yields (Mudau, 2006). The plants get nutrients from the soil, as well as from external nutrients sources, including fertilisers and organic manure (Maedza, 2015). Considering the increased biomass synthesis, adequate and proper nutrition is an important part of bush tea (Maedza, 2015). Tree growth is limited by many factors Mashimbye *et al.* (2006), lant development is limited by various factors (Mashimbye *et al.*, 2006); for example, when growth is limited due to lack of certain nutrients, the growth response to irrigation may be small (Lehlohonolo *et al*., 2013). Tea is a perennial plant that produces regular branches. Harvesting depletes the plant's nutrient reserves (Mudau and Ngezimana, 2014). Therefore, the use of fertilizers is important to improve soil nutrients (Mudau and Ngezimana, 2014). In research on the nutritional value of bush tea, many researchers have found that nitrogen (N), phosphorus (P) and potassium (K) can increase the height of the plant, its new and dry flowers, and many flowers of the bush tea branch (Maedza, 2015).

According to Joubert *et al*. (2008), bush tea grows best when there is 300 kg of nitrogen, 300 kg of phosphorus and 200 kg of potassium are applied per hectare. In a study on the seasonal nutrition of bush tea, all phenols increased with nitrogen, reaching a maximum of 51. 1 mg/g at a winter nitrogen concentration of 200 kg/ha N (Mudau *et al.*, 2007b). The results of the phosphorus study showed that the total phenolic content increased to 46.8 mg/g at 300 kg/ha in the winter (Mudau *et al.*, 2007a). The total polyphenol content is generally between 0 and 200 kg/ha K, which can be used as a good value for N, P and K application (Chabeli *et al*., 2008).

2.9.2 Dry matter content

The effects of various management and environmental factors on product weight, dry matter and product content have been examined in many studies (Mudau, 2006; Ng'etich and Stephens, 2001). The planting date is another element that influences dry matter (Mudau, 2006). The dry matter of tea, which gives it its nutritional value and health advantages, is made up of polyphenols, proteins, carbs, alkaloids, amino acids, pigment, vitamins, and minerals, among many other substances (Mudau *et al*., 2016). Throughout the production process, dry matter is an important factor that determines the quantity and quality of tea; However, traditional methods for determining dry matter content are very time consuming (Mudau *et al*., 2016).

The main content in shrub leaves is represented by dry content, which is important in determining plant tissue (Maudu *et al*., 2012). Previous studies have shown that there is a positive correlation between dry matter content, harvest time, and average g/tree yield of cultivated bush tea, while the correlation between wild bush tea is weak (Mudau *et al*., 2016). According to research conducted in South Africa, bush teas are grown in the autumn (Autumn), winter months (June, July and August) and have dry spots in the spring (September, October and November), followed by summer months (December, January and February) (Mudau *et al*., 2016). According to Mudau *et al*. (2016), Bush tea grown in the field has the highest drying rate in the summer months (December, January and February), and the lowest drying rate in the winter months (June, July and August) due to cold weather (Mudau *et al*., 2016).

Additionally, tea plants grown in greenhouses showed higher dry matter content (Maudu *et al*., 2011). This is a result of high temperatures, especially at night, which affect photosynthesis and increase respiration rate (Maudu *et al*., 2012). According to a study by Maudu *et al*. (2011); Mudau *et al*. (2016), detected the highest carbohydrate content in the field and the lowest carbohydrate content in the greenhouse environment. This shows that during the summer different parts of the plant distribute the percentage of total dry matter in different proportion of dry matter of bush tea (Tshivhandekano *et al*., 2018).

2.9.3 Starch content

According to Mudau *et al*. (2016), starch is a carbohydrate containing many sugars as a way to store energy (Marasha *et al*., 2013). Starch and soluble sugar in the samples were detected using the perchloric acid method (Marasha *et al*., 2013). According to research conducted on tea plants in South Africa, sugar levels are high in winter and low in summer (Mudau *et al*., 2016). Similarly, carbohydrate reserves in bush tea stems are lowest in summer and autumn, followed by winter (Mudau *et al*., 2016). Moreover, in terms of the seasonal response of carbohydrates in bush tea leaves, this season has the lowest starch compared to other seasons (Mudau and Makunga, 2018). As a result, the sugar content in winter is highest in winter and summer and lowest in autumn; The season in which all carbohydrates are stored is highest in winter, followed by summer and spring, and lowest in autumn (Mudau and Makunga, 2018). Therefore, as mentioned earlier, many climatic factors can affect the starch content of crops, including temperature and daylight hours, which reduce photosynthesis and respiration, thereby reducing starch content (Mudau and Makunga, 2018). Temperature increases the evaporation rate due to the conversion of starch content in the stem to sucrose, affecting the mineral nutrition and metabolism of the plant, causing the sugar content to increase and the dry matter to decrease (Mudau *et al*., 2016).

2.9.4 Minerals and vitamins

The nutritional value of tea is limited to certain vitamins and minerals (Gruenwald, 2009), and few vitamins and minerals have been considered as major activities because the composition of minerals and vitamins ain tea varies depending on their locations in plants. Plant genotype, age and the environmental factors all affect the nutritional value of tea (Maudu *et al*., 2012). Since protein and vitamins are essential for human nutrition, total protein and vitamin composition is important in tea production. According to previous studies on the mineral composition of tea, potassium (K) is one of the most abundant elements in tea (Maudu *et al*., 2012). The polyphenolic components (flavonoids and phenolic acid) found in teas are strong antioxidants that are scavenge-free radicles and protect the plant against diseases and a good source of minerals (Maedza, 2015).

According to a South African research Maedza (2015), bush tea contains sizable levels of calcium (Ca) and magnesium (Mg). Black tea leaves are the highest source of all minerals, according to nutritional data (Maedza, 2015). Since the bush tea is the root of most rural groups, tea leaves are rich in phenolic antioxidants (Mudau, 2006). Busch tea leaves are rich in phenolic antioxidants as it is the most popular indigenous tea among rural people (Mudau, 2006). According to research, dried leaf samples of coca tea and bush tea have the highest mineral content (Mudau, 2006). For this reason, the potassium content of all other teas is higher than other minerals (Ismail *et al*., 2000). Bush tea is the most mineral-rich indigenous plant, which is known to affect the quality of beverages (Mudau, 2006).

2.9.5 Metabolomics

Metabolomics, as defined by Hasanpour *et al*. (2020) and Ch *et al*. (2020), is a large study of small molecules found in cells, biological fluids, tissues, and organisms (also known as metabolites). The collection of small molecules and their intracellular interactions is called an “organism's metabolome”. According to Pirhaji (2016), the study of the metabolite composition or metabolome of a type of cell, tissue, organ, or organism is called metabolomics. Metabolomics provides a “snapshot” of the biological significance of the process by measuring the global collection of low molecular metabolism such as amino acids, organic acids, sugars, fatty acids, lipids, steroids, short peptides, and vitamins (Pirhaji, 2016). It allows reading the metabolic activity status in relation to genetics, gene expression or the environment (Pirhaji, 2016). These external stimuli include infections and allergies.

Gene-environment interactions between environmental factors and host molecules such as proteins, lipids, and other enzymes are defined by specific metabolome profiles (Sánchez *et al*., 2020 Drugs made from natural products (NPs) are analysed and their quality evaluated using the powerful metabolomics technique (Lee *et al*. 2017). According to Glauser *et al*. (2013), molecular networks (MN) facilitate the identification of structural connections. The spectral library obtained from the Molecular Network (MN) shows that compounds with similar structures were detected (Wang *et al*., 2021). Plants are useful as food and contain many natural product and phytochemicals they contain (Glauser *et al*., 2013). Bush tea contains chlorogenic acid, which helps improve cardiovascular health. However, the are few reviews of the bush tea metabolomes (Ramphinwa *et al*., 2022).

2.9.6 Metabolomics experimental approach and pipelines

Untargeted (global) and targeted techniques are classifications of metabolomics experiments. These methods differ in many aspects, including degree of quantification (Carneiro *et al*., 2019 Untargeted metabolomics is the objective analysis of the metabolite profile of biological products in specific situations in the body under environmental conditions (Carneiro *et al*., 2019). It is difficult to objectively cover all metabolites due to limitations of available measurements, obtained samples, and environmental performance. However, the non-target method can be considered as a target since no metabolites are detected before sample analysis. The understanding the metabolite class of the nervous system can help select appropriate analytical platforms and models to improve the detection of metabolites (Carneiro *et al*., 2019).

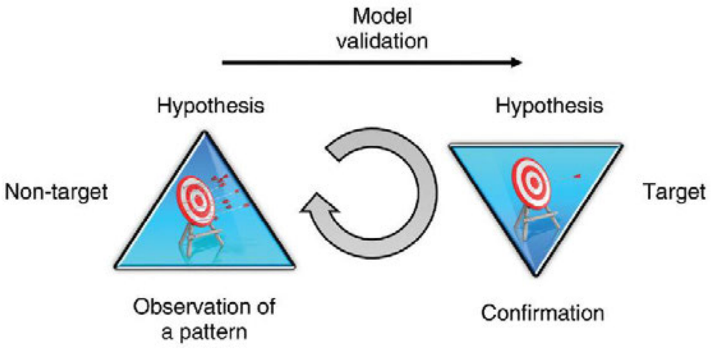

Figure 3: Metabolomics approach (Carneiro et al., 2019)

One of the several techniques used in molecular networking is the Triple Quadrupole Mass Spectrometer (TQMS) (van Outersterp, 2023). Pipelines, as shown Figure 3, facilitate sample preparation, data collection, and analysis. They also facilitate the separation and characterization of different groups of metabolites (Henderson *et al*., 2016).

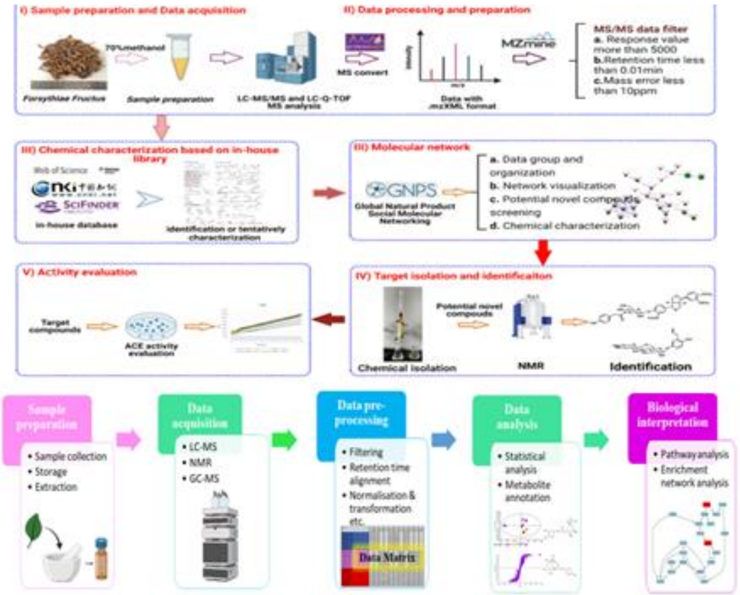

Figure 4: Metabolomics pipelines (Li et al., 2022)

2.10 Plant Water Relations

t is reported that the relationship between tea and water has been studied by many researchers at three research centers in East Africa (Carr, 2001). Better understanding of the response of plants to their environment, management in the area and methods for early identification of drought-tolerant diseases, before measuring stomatal behavior, photosynthesis, transpiration and xylem water potential (Carr, 2001). Therefore, the following shall examine the nutritional water productivity:

2.10.1 Nutritional Water Productivity (NWP)

Nutrient water productivity (NWP) is defined as a measure of yield and nutrient production per unit of water used (Wenhold *et al*., 2007). Food security accessibility, affordability and implementation issues can be addressed through aquatic nutrition (Ong *et al*., 2007). Climate forecasting has been shown to be a useful strategy for integrating water, agriculture and food supply (Ong *et al*., 2007). Energy per unit of water, calcium, fat, vitamin A and iron are factors that affect the nutritional value of water (Selvaratnam *et al*., 2003). Recently, two indicators have been developed; water projects and water quality improvement to assess the impact of food and economic growth in water-stressed regions, particularly in Africa (Selvaratnam *et al.*, 2003). In a semi-arid tropical region including Africa, where water is scarce and food is becoming increasingly unstable, aquatic foods are said to be a better strategy (Wenhold *et al*., 2007). It is considered a finite and limited natural resource to increase the amount of food (Carr, 2001).

In a planting density trial in Tanzania, technology-based fluid flow measurement was successfully used to determine disease spread and represent results from agricultural water production (Descheemaeker *et al*., 2010). Most rural farmers in sub-Saharan Africa cannot afford inputs that can increase productivity, such as pesticides and herbicides (Descheemaeker *et al*., 2010). Water is essential for improving nutrition and human health (Rockström *et al*., 2009). In addition, crop scientists continue to develop agricultural techniques that address water issues and combine more efficient management techniques, while growers are working to grow and develop water-efficient crops (Carr, 2001). However, although the increase in water production is thought to be beneficial, the problem remains unsolved because of water shortage (Carr, 2001).

2.10.2 Crop water requirements

The amount of water required to cover the evaporation and transpiration loss from the crop field at a certain time is called crop water requirement (CWR) (Ali, 2010). Moreover, crop water requirements are usually given in millimetres on a daily, monthly or seasonal basis (Chiarelli *et al.*, 2020). They are also utilised for management purposes, in terms of irrigation water estimate irrigation and water delivery needs, as well as irrigation and water delivery schedules (Ali, 2010). Because both terms relate to the same amount of water, crop water requirements and crop evapotranspiration are strongly intertwined (Surendran *et al*., 2015); however, there is a distinction between them (Mehta and Pandey, 2016). According to Gong *et al*. (2020), state that this water is actually similar to the water quality system that should be used to achieve good results. Crop evapotranspiration and then water need to be calculated first, as the latter generally present the effect of crop evapotranspiration over time (Mehta and Pandey, 2016).

To estimate water demand and control water use, crop evaporation needs to be converted into crop water demand (Gong *et al.*, 2020). With the development of large-scale irrigation projects, the concept of agricultural water demand has gained importance, making it necessary to estimate water use in newly irrigated areas (Gong *et al.*, 2020). The crop water requirements (CWR) for current management often differ from the long-term planning, where the CWR can be estimated using near-season climate data (Sawant *et al*., 2017). Therefore, further data on the measurement and estimation of crop water requirements of the *Athrixia phylicoides* DC. are limited (Surendran *et al*., 2015).

2.10.3 Climate factors influencing the crop water requirements

Crops grown in hot and cold areas need more water per day than crops grown in cool areas (Li *et al*., 2020). In addition to sunlight and temperature, there are other climate or variables that affect crop water (Molua and Lambi, 2006), two of which are humidity and wind speed (Savva and Frenken, 2002). Crops require more water in drought than in wet conditions (Molua and Lambi, 2006), while crops in windy climates consume more water than those in calm regions (Li *et al*., 2020). The largest crop water requirements are consequently found in hot, dry, windy and sunny climates (Savva and Frenken, 2002). When the weather is chilly, humid and gloomy, with little or no breeze, the lowest readings are obtained (Li *et al*., 2020). Obviously, the water needs of crops grown in different climates will be different (Savva and Frenken, 2002).

2.10.3 Crop management

According to Mudau *et al*. (2005), crop management is a process that begins with planting, continues with crop management during growth and development, and ends with harvesting, storage and distribution of the crop. It has long been known that crop management affects pests and their natural enemies in the soil environment (Mudau *et al*., 2005). According to Mudau *et al*. (2006), state that crop management affects plant residues and pesticides in the soil and has an impact on the soil environment and microbial growth. In addition, herbal tea also requires good crop management with a good environment, fertile soil and sufficient water to support the growth of healthy plants (Tshivhandekano *et al*., 2013).

2.11 Conclusion

In many parts of Southern Africa, including Limpopo, KwaZulu-Natal and Mpumalanga, smallholder farmers often cultivate the bush tea for its medicinal properties. Many studies by many scientists have identified the specific soil and environmental conditions required for good tea cultivation; These include many factors, such as high humidity and acidic soil found in some regions, especially Asia and Africa. However, significant research gaps have been identified hindering progress in the bush tea industry in South Africa. Therefore, this review shows limited information on tea plant water use and water production (including yield and quality). More importantly, irrigated food production has become important, especially on medicinal plants to improve human health. More research is needed to provide general recommendations for farmers to improve crop yield and quality by using water correctly when growing bush tea. Farmers need to pay attention to important factors such as planting date, fertilization, variety selection. Additionally, this study reveals the importance of planting date and fertilization in terms of bush tea yield and quality. Farmers are encouraged to follow prescribed pesticides and follow safety recommendations between application and harvest. Additionally, addressing the mystery and understanding the physical forces that can limit the quality of the bush tea is important for the cultivation and further use of the bush tea plant.

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CHAPTER THREE

RESPONSE OF PHYSIOLOGICAL PARAMETERS, NUTRITIONAL WATER PRODUCTIVITY, AND PHYTOCHEMICAL PROFILES TO VARYING WATER REGIMES OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.)

Muneiwa Rumani[1*], Tafadzwanashe Mabhaudhi[2,3], Anza-Tshilidzi Ramabulana[4], Ntakadzeni Edwin Madala[4], Maanea Lonia Ramphinwa [5], Lembe Samukelo Magwaza[1,6], Fhatuwani Nixwell Mudau[1*]

[1]School of Agricultural Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa
[2]Centre of Transformative Agricultural and Food Systems, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa
[3]International Water Management Institute (IWMI-SA), South Africa, Pretoria, South Africa
[4]Department of Nutrition, Faculty of Health Sciences, University of Venda, Private Bag X5050, Thohoyandou, Limpopo 0950, South Africa
[5]Department of Plant and Soil Science, Faculty of Sciences, Agriculture and Engineering, University of Venda, Private Bag X5050, Thohoyandou, Limpopo 0950, South Africa
[6]Department of Food Systems and Development, Faculty of Natural and Agricultural Sciences, University of Free-State, PO. BOX 330, Bloemfontein 9300, Republic of South Africa

*Corresponding author: muneiwaabigail@gmail.com; mudauf@ukzn.ac.za

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Abstract: Incorporating the water-food-nutrition-health plays a significant role in advancing the cultivation of *Athrixia phylicoides* DC., (bush tea) in Sub-Saharan Africa (SSA) to alleviate food and nutritional insecurity by considering physiological parameters, nutritional yield (NY), nutritional water productivity (NWP), and metabolite composition. The primary aim of the study was to determine the physiological parameters, nutritional yield, NWP, and metabolite content of bush tea under varying water regimes. The controlled experiment was laid out in a complete randomized block design (CRBD), treatments consisted of three water regimes (100%, 30%, and control (stress)) of the crop requirements (ET_a), replicated three times. The physiological parameters were taken weekly. However, yield, nutrient content, nutritional water productivity (NWP), and phytochemical analysis were determined at harvest. The phytochemical analysis by liquid chromatography-mass spectrometry (LC-MS) coupled with visualization of the detected chemical spaces through molecular networking indicated *Athrixia phylicoides* DC. to be rich in various bioactive compounds derivatives. The results show that the 30% ET_a enhanced the plant growth, nutrient content, and nutritional water productivity compared to other water treatments. Nevertheless, 100% ET_a water treatment (95.62 $kg.ha^{-1}$) yielded more than 30% ET_a water treatment (60.61 $kg.ha^{-1}$) and control (12.12 $kg.ha^{-1}$). The accumulation of bioactive compounds was greater when subjected to 30% ET_a water treatment in comparison to both 100% ET_a and control water treatments. Therefore, this study is the first to determine the accumulation of various bioactive compounds in bush tea leaf extracts under varying water regimes. This confirms that in areas with low water availability, bush tea is well adapted for production without limiting nutrients.

Keywords: herbal tea, water treatments, water productivity, nutrient content, water use efficiency, growth development, yield, molecular network

Introduction

Bush tea is a South African nutritious herbal that is used as medicine by many households (Joubert *et al*., 2008). Traditionally, it is well-known as an herbal tea that purifies blood, treats boils, headaches, infested wounds, and cuts, and creates a foam bath (Mabogo, 1990; Mudau *et al*., 2007; Tshikhudo *et al*. 2020). Its roots are used as an aphrodisiac by the Vhavenda people (Rakuambo, 2007) while a decoction of the roots is used by the Zulu people as a cough suppressant and laxative (Tshivhandekano *et al*., 2013). Tshivhandekano *et al*. (2013) and Lerotholi *et al*. (2017), stated that the plant tissues contain a significant number of bioactive compounds.

Despite its medicinal properties, the tea plant can grow naturally at various altitudes and with different rainfall and soil properties (Tshikhudo *et al*., 2019). Seasonal changes in temperature and vapor pressure will affect plant growth, yield, and bush tea quality (Ramphinwa, 2023). Gatabazi *et al*. (2022), reported that cultivation and climate change affect the life cycles of medicinal plant species and their distribution in different regions. Therefore, a controlled environment is one strategy to mitigate the changes in agriculture.

South Africa is recognized as a water scarcity nation (Chivenge *et al*., 2015). The annual average rainfall in the country is approximately 500 mm, which is lower than the world average of 860 mm (Mabhaudhi. 2012). In addition, Bendou (2022), found that one of the main variables limiting plant development and productivity in agricultural production for different crop species is dryness. In addition, drought affects plants at various stages of development, from fertilization through harvest, resulting in decreased plant development (Chrysargyris *et al*., 2018). Therefore, it is critical to determine the effect of cultural practices such as varying water regimes under a controlled environment to produce high-quality bush tea production.

Previous research on bush tea production has investigated plant growth, yield, and diverse cultural techniques such as nutritional management in a controlled and field environment (Mudau *et al*., 2006), pruning (Mohale *et al*., 2018), harvesting approaches (Mphangwe, 2012), processing (Hlahla, 2010) and seasonal variation (Mudau *et al*., 2007; Ramphinwa *et al*., 2022). However, the impact of bush tea under different water regimes on growth, yield, and secondary metabolites in a controlled environment is still lacking. Irrigation is a significant factor that affects the growth productivity and quality of medicinal crops (Hassan and Ali, 2014). The tea plant, *Camellia Sinensis* (tea) responds well to irrigation and thrives well in dry land conditions (Carr, 2010). An important factor affecting crop yield and nutrient loss is the water used by crops during the growing season and water loss or vegetation (as evapotranspiration) (Dasila *et al*., 2016). Consequently, the study aimed to investigate the responses of physiological parameters, nutritional water productivity, and phytochemical analysis of bush tea grown under a controlled environment to varying water regimes.

Material and methods

Experimental site. The protected environment experiment was conducted from the spring to summer season of the year 2022 in a tunnel situated at the University of KwaZulu-Natal's Controlled Environment Research Unit (CERU) (29.58° S, 30.42° E), in Pietermaritzburg, South Africa. Weather data was sourced from the University of KwaZulu-Natal (UKZN) agrometeorology weather service for the spring to summer season of the year 2022. The temperatures ranges from ~ 18/33 °C (day/night) and relative humidity (60-80%).

Plant material description. Bush tea stem cuttings were collected from the wild in Pietermaritzburg, Midlands (lat. 29˚.6168'S long. 30˚.3928'E), South Africa. A length of 7 to 8 cm of stem cuttings were dipped in Seradix No. 2 hormone (0.3% IBA) to stimulate the rapid and abundant root formation process. Cuttings were then grown on a 30 cm long plastic bag in a tunnel. A daily irrigation system was used on the plants. To prevent transplanting shock, the rooted cuttings were transplanted into 1L bags after 35 days for three months (Maedza, 2015). Thereafter, after being stored for 40 days, planting materials with 25 leaves, were transplanted into long-raised beds.

The experiment was conducted on raised beds with a set width of 0.75 m and a length of 11 m. Thereafter, uniform plants were chosen for the trial layout of water treatments. Planting beds were prepared before establishing a trial. Regular hand weeding was done to keep the experiment pest-free. Use karate (30 mL/15 L water) twice a week for eight weeks after planting to control the disease. The soil was submitted to the Institute for Commercial Forestry Research (ICFR) Analytical and Research Laboratory to determine chemical and physical properties. Therefore, the soil texture was clay Table 1. However, soil tests Table 1 show that the soil needs nitrogen fertilizer which was applied in the soil with a concentration of 4 g of limestone ammonium nitrate (LAN) based on recommendation by Mudau *et al*. (2007a). Weather data was sourced from the University of KwaZulu-Natal (UKZN) agrometeorology weather service for the spring to summer season of the year 2022.

Table 1: Physical and chemical properties of soil in the long-raised beds

Clay (%)	**Organic C (%)**	**pH (KCI)**	**P (cmol/kg)**	**Ca (cmol/kg)**	**K (cmol/kg)**	**Mg (cmol/kg)**	**Na (cmol/kg)**	**N (%)**
38.5	2.70	5.19	539.62	1059	0.36	1.11	0.23	0.19

Experimental description. The experiment was laid out in a complete randomised block design (CRBD) with three water requirements (100%, 30%, and control (stress) replicated three times. Water flow was supplied with a filter, a pump, two solenoid valves, two water meters, a control box, Netafim inline drippers with four individual drippers, and a mainline that supplies water three times a day at 7, 11, and 3 a.m. The average rate per dripper was 2 Lhr^{-1}, while the drip rate was operating at 33 ml per min^{-1}, whereas the system's maximum working pressure was set to 200 kPa. The discharge rate was set at 2 Lhr^{-1}. The irrigation event and irrigation interval were the same. However, water was provided twice daily at 7 and 11 a.m. with a discharge rate of a total of 2 L per day using a watering can for control (stress) for over 40 days after transplanting, thereafter, irrigation was stopped to stress the bush tea. The spacing was 0.5 m x 0.5 m (inter and intra row spacing) (40 000 plants/ha $^{-1}$ plant population). Because the crop factor for bush tea has not yet been identified, the irrigation plan was created using the daily crop water needed from the product of normal tea (unshaded) crop factors (K_c) as provided by (Singh, 2013) and the monthly average reference evapotranspiration (ET_o), because the crop factor is not yet determined on bush tea. Evaporation data were collected from the agricultural meteorology automatic weather station Table 2. All treatments were irrigated to the potential area before treatment application to reduce evaporation and runoff losses during peak sun hours and to ensure adequate soil moisture. Therefore, the crop water need was calculated using Eq. 1:

$$ET_a = ET_o \times K_c \qquad (1)$$

Table 2: Crop water requirements of bush tea in responses to varying water treatments

	K_c	**ET_o**	**ET_a**	**Duration**	**Total water applied**
		mm	mm	Days	mm
Initial	0.95	4.8	4.56	40	182.40
Mid-season	1.00	6.57	6.57	51	335.07
Late season	1.00	7.89	7.89	25	197.25
Total water applied (ET_a)					
• **100%**					714.72
• **30%**					214.416
• **0%**					0

Where: K_c=crop factor based on Allen et al., (1998), ET_a= crop water requirement (mm). ET_o = reference evapotranspiration (mm). and K_c = crop factor.

Data collection.

***Gas exchange and chlorophyll fluorescence measurements*.** Gas exchange and chlorophyll fluorescence were performed by LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) integrated with an infrared gas analyzer (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). The leaf was maintained at a temperature of 25 ˚C. The relative humidity was kept at 43% and the water flow rate was kept at 500 mol. In the cuvette, the leaf-to-air vapor pressure deficit was maintained at 1.7 kPa to prevent stomatal closure from low air humidity. Between 8:00 and 11:00 a.m., measurements of the third partially developed leaf from the plant's tip were made by placing the leaf within the detector head while also applying 100%, 30%, and control (stress) water treatments. Three plants per replication were used for taking measurements. Stomatal conductance (g_s), net CO_2 assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (C_i), and the ratio of intercellular and ambient CO_2 concentrations (C_i/C_a) were measured for gas exchange. The intercellular CO_2 concentration (A/C_i), which measures the net CO_2 assimilation rate. Calculating intrinsic water use efficiency (WUE_i) and instantaneous water use efficiency (WUE_{ins}) was done using the ratio of A and g_s

and A and *T*, respectively. According to Evans (2009), estimated the following additional chlorophyll fluorescence measurements: *Fv'/Fm'*. the maximum quantum efficiency of photosystem II photochemistry, the effective quantum efficiency of photosystem II photochemistry (*ΦPSII*), photochemical quenching (*qP*), non-photochemical quenching (qN), and electron transport rate (*ETR*), The ratio of *ETR* and *A* was used to calculate a relative measure of electron transport to oxygen molecules. To determine a relative indicator of electron transport to oxygen molecules, the ratio of alternative electron sink (*AES*) and *A* was utilized. The ratio of the quantum efficiency of CO_2 assimilation (*A*) to the effective quantum efficiency of the photosystem.

Yield. Bush tea plants were harvested 153 days after planting. Bush tea yield components were determined by sampling 3 plants in each replicate of each treatment. The yield (fresh below-ground mass), number of roots per plant, and number of marketable roots were all recorded on a plot-by-plot basis (Ossom and Rhykerd, 2008). Marketable roots were defined as entire (undamaged), free of harvest wounds, pest damage, and disease damage. Actual yield (Ya) was calculated using the fresh mass of marketable roots and then converted to $kg.ha^{-1}$. The percentage of marketable storage roots was calculated using Eq. 2:

$$\frac{Number\ of\ marketable\ roots/plot}{Total\ number\ of\ roots\ /plot} \times 100\% \qquad (2)$$

Determination of nutritional composition (NC). Bush tea leaves were dried in an oven at 40 ˚C for 24 hours and then ground into powder using a mortar and pestle. The ground samples (0.5 g) were placed in a quartz crucible and charred in a wild Barfield muffle furnace at 650 ˚C for 120 minutes. Acid digestion was carried out according to Huang *et al.* (2007) with minor modifications. The ash was dissolved in 10 ml of diluted aqua regia (HNO^3 and HCL mixed in a ratio of 1:3) in a 25 ml volumetric flask. The contents of the flask were brought to volume by adding distilled H_2O and heated in a water bath for 1 hr^{-1} at 85 ˚C. The samples were chilled to 25 ˚C before analysis using atomic absorption spectrometry (AAS) (Mandizvo and Odindo. 2019). The nutrients analysed per dry matter basis included micro-nutrients (calcium, zinc, iron, magnesium, and sodium).

Determination of nutritional water productivity (NWP). The productivity of nutrient waters was calculated using the formula of Renault and Wallender (2000) which was calculated using Eq. 3:

$$\mathrm{NWP} = \frac{\mathrm{Ya}}{\mathrm{ETa}} \times \mathrm{NC} \qquad (3)$$

where: NWP is the nutrient productivity of water (nutritive value m^{-3} of water evapotranspiration), Y_a is the actual harvested yield ($kg.ha^{-1}$), ET_a is the water applied based on crop water requirement ($m^3.ha^{-1}$), and NC is the nutrient content per kg of product (nutritive value kg^{-1}).

Metabolite extraction. A method suggested by Makita *et al*. (2016) was used to determine the extraction of metabolites. The tea leaves were freeze-dried and then ground into a fine powder. The 2 grams (g) of powder was extracted with 20 ml (1:10 m/v) of 80% aqueous methanol (Romil SpS, Cambridge, UK). The samples were rotated at 70 rpm in a digital rotisserie tube rotator for the whole night. Thereafter, the homogenate was centrifuged at 5000 gn to remove debris, for 20 minutes. The supernatant was subsequently filtered using 0.22 µm nylon filters into glass vials with 500 µL inserts. For each sample group, at least, 3 independent replicates were prepared, these were stored at 4 ˚C (Ramabulana *et al*., 2021). Equal quantities of each sample were mixed to generate quality control (QC) samples, which were used in conjunction with experimental samples to evaluate the quality of the data collected (Ramabulana *et al*., 2021).

Liquid Chromatography-Quadruple Time-of flight Tandem Mass Spectrometry (LC-MS/MS). A Liquid Chromatography-quadruple time-of flight tandem mass spectrometry instrument (QTOF)–MS, model LC-MS 9030, Shimadzu Corporation, Kyoto, Japan). The chromatographic separation was performed on a Shim Pack Velox C18 column (100 mm × 2.1 mm with particle size of 2.7 µm) was used to analyse the prepared bush tea leave extracts. The column oven was set at 40 ˚C as described by (Ramabulana *et al*., 2021). A binary solvent gradient, consisting of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used at a constant flow rate of 0.4 mL/min. The gradient was set for 53 minutes, and the flow rate was set at 0.3 mL/min under the following separation conditions: The settings were maintained at 10% B for 3 min, 10%–60% B over 3–40 min, 60% B from 40–43 min, then the gradient increased to 90% B between 43–45 min, and kept at 90% B for 3 min. The gradient was reset to its initial state, 48–50 minutes later. This was followed by a 3-min column re-calibration period. A mass spectrometer detector was used for monitoring analyte elations, under the following conditions: ESI (electrospray ionization) negative modes; interface voltage of 3.5 kV; nitrogen gas was used as nebulizer at flow rate of 3 L/min, heating gas flow at 10 L/min; heat block temperature at 400 ˚C., CDL temperature at 250 ˚C, detector voltage of 1.70 kV and the TOF temperature at 42 ˚C. Tandem MS (MS/MS) experiments, typical mass accuracies with a mass error below 1 ppm was obtained using a mass calibration solution of sodium iodide (Nal). High resolution was obtained for MS and (MS/ MS) experiments using a mass-to-ratio (m/z) range of 100-1000 Da.

Molecular networking. For all datasets acquired by Table 3, the network was constructed using the Wang *et al*. (2016) suggested technique and the online Global Natural Products Social Molecular Networking (GNPS) process (https://gnps.ucsd.edu). The data was filtered by removing all MS/MS fragment ions that were within +/- 17 Da of the m/z precursor. Only the top 6 fragment ions within the +/- 50 Da window were selected from the window-filtered MS/MS spectra. The MS/MS fragmentation ion tolerance (MS/MS) was set at 0.5 Da, while the mass tolerance for the precursor ion was 2.0 Da. Following the recommended procedure by Aron *et al*. (2020), a network with edges with cosines larger than 0.7 and more than 6 associated peaks was produced. Additionally, edges detected between two nodes were preserved in the network when both nodes occurred in their 10 most similar nodes. The maximum molecular family size was eventually set at 100 and the lowest-ranked edges were removed from the molecular families until the molecular family size fell below this threshold. Then the GNPS spectral libraries were used to search for network spectra. The library spectra were filtered in the same way as the input data. All matches between the library and lattice spectra are required to contain at least 6 matching peaks and a value larger than 0.7 (Aron *et al*., 2020).

Data analysis. Data were subjected to analysis of variance (ANOVA) using GenStat® version 20 (VSN International. UK). The least significant difference (LSD) was used to separate means at the 5% significance level. Principal component analysis (PCA) was per-formed for all metabolites and the molecular network analysis were constructed on the GNPS while the raw data were acquired from the Shimadzu LCMS-9030 QTOF of bush tea extracts under varying water regimes.

Results and discussion

Gas exchange and chlorophyll fluorescence measurements. It is important to understand the physiological processes that lead to the development of adaptation to water in the production of tea plants. Water level significantly ($P<0.01$) affected the gas exchange and chlorophyll fluorescence measurement of bush tea Tables 3 and 4. The highest photosynthetic rate was recorded under 30% ET_a followed by 100% ET_a and control (stress) was recorded as the lowest. Similarly, stomatal conductance (gs), net CO2 assimilation rate (A), transpiration rate (T), intercellular CO2 concentration (Ci), ratio of intercellular and ambient CO2 concentrations

(Ci/Ca), intrinsic water use efficiency (WUEi) and instantaneous water-use efficiency (WUEins) increased under 30% ET_a compared to 100% ET_a and control (stress). The measured chlorophyll fluorescence measurements i.e., Fv'/Fm', maximum quantum efficiency of photosystem II photochemistry, effective quantum efficiency of photosystem II photochemistry (ΦPSII), photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR) varied significantly among the varying water treatments, with the highest values being recorded under 30% ET_a compared to 100% ET_a and control (stress). The results contradict those of Barros *et al.* (2023), who reported the reduction of net photosynthesis (A), stomatal conductance (gs), transpiration (E), and internal CO2 concentration (Ci) due to water stress. It is critical to understand how and when to apply limited amounts of water to maximize the yield of high-quality plant products (Alderfasi and Alghamdi, 2010). Bush tea may thrive well when plants are exposed to 30% ET_a water stress.

The evaluated bush tea exhibited high water use efficiency under limited water application (30% ET_a). These results suggest that bush tea is efficient water-users attributed to limited water application Table 4 as the study exhibited maximum WUE_i and WUE_{inst} compared to other water treatments. The study revealed that bush tea plants are capable of reducing loss of water when exposed to water stress. Efficient PSII activity under limited water application protects the structure of chloroplasts against oxidative damage by preventing the creation of singlet oxygen (Levesque-Tremblay *et al.*, 2009). Positive performance under limited water application is probably influenced by maintaining the effectiveness of PSII function and the antioxidant system components. The current study is the first to show that morphological measurements under limited water enhance the morphological development of bush tea under a protected tunnel environment.

Table 3: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence measurements of bush tea evaluated under varying water treatments

Source of variance	**d.f**	***gs***	***T***	***A***	***Ci***	***A/Ci***	***Ci/Ca***	Wu_i	WUE_{inst}
Water treatment	2	0.1401667**	10.5797**	3.47367**	61477.6**	6.63433**	376.711**	97.933**	175.599**
Time	1	0.14062678**	865.2420*	1509.6744**	535691.9**	1.82133**	2332.305**	2055.305**	18203.825**
Water treatment x time	2	0.00019671*	1.9300*	0.3664**	12523.9**	43.27496**	339.606**	2.060**	3.707**
Residual	12	0.0003317	0.1835	0.05408	255.2	0.06533	1.119	1.536	2.355
Source of variance	**d.f**	**Fo**	**Fm**	**Fv/Fm**	Φ_{PSII}	**qP**	**qN**	**ETR**	
Water treatment	2	197228**	390037**	0.1698479**	352.197**	71.56132**	78.52056**	1853447**	
Time	1	232382**	570074*	0.1557303*	843.243**	119.91842**	81.06889**	6754541**	
Water treatment x time	2	5079*	7197*	0.0007281*	76.338**	19.88921**	10.57056**	831555**	
Residual	12	1514	3333	0.0003534	2.808	0.04054	0.07444	1342	

d.f; degrees of freedom, **g_s**; stomatal conductance, **T**; transpiration rate, **A**; net CO_2 assimilation rate, **A/C_i** ; CO_2 assimilation rate/intercellular CO_2 concentration, **C_i**; intercellular CO_2 concentration, **C_i/C_a**; ratio of intercellular and atmospheric CO_2, **WUE_i**; intrinsic water use efficiency, **WUE_{ins}**; instantaneous water-use efficiency, **F_v/F_m**; maximum quantum efficiency of photosystem II photochemistry, **Φ_{PSII}**; the effective quantum efficiency of PSII photochemistry, **qP**; photochemical quenching, **qN**; non-photochemical quenching, **ETR**; electron transport rate, **ETR/A**; relative measure of electron transport to oxygen molecules, ***** and ****** denote significant at 5 and 1% probability levels, respectively, **Ns**; non-significant.

Table 4: Means of leaf gas exchange and chlorophyll measurements of bush tea under varying water treatments

Leaf gas exchange measurements										**Chlorophyll Fluorescence measurements**							
Week1	**ET$_a$**	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control (no irrigation)	0.18a	32.48a	26.27a	318.0a	3.40a	0.85a	20.83a	52.30a	2676.4a	2437a	0.1219a	20.10a	0.137a	1.13a	663.5a	22.67a
	100%	0.37b	35.01b	28.30b	330.5b	3.56b	0.94b	26.51b	60.23b	2803.0b	2657b	0.250b	24.52b	2.10b	3.30b	943.6b	31.13b
	30%	0.3487c	36.18c	29.74c	423.9c	6.83c	1.59c	34.10c	64.22c	2983.6c	2951c	0.440c	28.96c	3.41c	6.133c	1036.6c	33.72c
Leaf gas exchange measurements										**Chlorophyll Fluorescence measurements**							
Week2	**ET$_a$**	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUEi***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***	***ETR/A***
	Control (no irrigation)	0.35a	47.63a	29.63a	570.9a	5.53a	11.99a	38.94a	117.68a	2837.8a	2848a	0.30a	25.56a	1.20a	2.367a	1205.7a	25.31a
	100%	0.56b	48.45b	30.45b	677.2b	8.67b	18.34b	40.86b	122.63b	3051.5b	2935b	0.42b	42.76b	8.50b	9.267b	2058.0b	42.47b
	30%	0.65c	49.19c	31.19c	877.2c	1.5c	41.35c	42.75c	127.25c	3255.4c	3330c	0.65c	46.32c	11.43c	11.667c	3055.5c	62.12c

d.f; degrees of freedom, **g_s**; stomatal conductance, **T**; transpiration rate, **A**; net CO2 assimilation rate, (µmol CO2 m^{-2} s^{-1}), **A/C_i**; CO2 assimilation rate/intercellular CO2 concentration, **C_i**; intercellular CO2 concentration, **C_i/C_a**; ratio of intercellular and atmospheric CO2, **WUE_i**; intrinsic water use efficiency, **WUE_{ins}**; instantaneous water-use efficiency, **F_v/F_m**; maximum quantum efficiency of photosystem II photochemistry, **Φ_{PSII}**; the effective quantum efficiency of PSII photochemistry, **qP**; photochemical quenching, **qN**; non-photochemical quenching, **ETR**; electron transport rate, **ETR/A**; relative measure of electron transport to oxygen molecules. Varying upper-case letters within a column indicates significant differences among water treatments. **g_s**; (mmol m^{-2} s^{-1}), **T**; (mmol H2O m^{-2} s^{-1}), **A**; (µmol CO2 m^{-2} s^{-1}**)**, **A/C_i**; (µmol. mol m^{-1}), **C_i**; (µmol. mol m^{-1}), **WUE_i**; (µmol (CO2) m^{-2}); **WUE_{ins}**, (µmol. mol^{-1}), Fv/Fm; (ratio); **ΦPSII**, the effective quantum efficiency of PSII photochemistry; **q_P**, photochemical quenching; **q_N**, non-photochemical quenching; **ETR**, (µmol e^{-1} m^{-2} s^{-1}); **ETR/A**, (µmol e $µmol^{-1}$ CO2).

Yield. Varying water regimes had significantly affected (P<0.01) yield in bush tea production Table 5. The yield metrics of bush tea plants subjected to various water regimes and grown in a controlled environment differed significantly. The higher yield was recorded under 100% ET_a (96 kg. ha^{-1}) compared to 30% ET_a (60.6 kg. ha^{-1}) and control (stress) (12.12 kg.ha^{-1}). The findings could imply that crop yield increases as irrigation water supply increases (Fereres *et al*., 2003). The results are like those reported earlier by Mabhaudhi *et al*. (2018), who also had similar water regimes on sweet potato production. A higher number of roots were recorded on bush tea plants that were exposed the 100% ET_a (46 kg) than plants from 30% ET_a and control (stress). The percentage of marketable roots considered differently between the water levels, with 100% ET_a recording the most marketable roots, followed by 30% ET_a, and with the lowest marketable roots recorded under control (stress condition). These results are similar to those of (Osman, 2000; Hassan and Ali, 2014), who found that more water leads to better growth and better outcomes compared to lower levels. Higher yield may be due to the full application of water to bush tea which promotes vegetative growth, yield, and quality (Hassan and Ali, 2014). However, 30% ET_a had significantly (P<0.01) higher water productivity than 100% ET_a and control (stress). This result could have been attributed to the volume of water consumed by bush tea plants when exposed to 30% $ET_{a,}$ because WP depends on the amount of the output (Ali and Talukder, 2008). Thus, the results of the current study are similar to the findings by Mabhaudhi *et al*., (2018); Mabhaudhi. (2012); Motsa *et al*., (2015).

Table 5: Yield and yield components of varying water treatments (100% ET_a, 30% ET_a, and control (no irrigation)

Water treatments (ET_a)	**Yield (kg/ha)**	**Number of roots**	**Marketable roots (%)**	**WP (kg/m^3)**
Control	12.12a	15.33a	13.33a	0a
30%	60.61b	32.67b	29b	95.62b
100%	95.62c	45.67c	41.67c	202.03c
$LSD_{(p < 5\%)}$	0.02	0.04	0.02	0.01

Water productivity (WP) represents the total biomass. The yield, number of roots, marketable roots and WP values are statistically significant with p-values < 0.001, according to the LSD (5% level).

Elemental composition. Nutrient components of bush tea exposed to (30%, 100% $ET_{a,}$ and control (stress) of the crop water requirements differed significantly ($P<0.05$). The highest nutrient compositions (calcium, copper, iron, potassium, magnesium, manganese, and zinc) were recorded under 30% ET_a, followed by 100% ET_a, with the lowest elemental composition recorded under control (stress) Table 6. The study revealed that bush tea may generate more nutrients when exposed to 30% ET_a. This observation aligns with previous study by Mabhaudhi et al., 2018, who demonstrated the influence of water availability on nutrient uptake and plant physiology on potato cultivars. Additionally, the results highlight the importance of water management strategies in enhancing the nutritional quality of bush tea plants.

Table 6: Elemental composition of bush tea with varied water treatments (30% ET_a, 100% ET_a and control (no irrigation) of crop water requirements)

Water treatments (ET_a)	**Ca (mg/L)**	**Cu (mg/L)**	**Fe (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**Mn (mg/L)**	**Zn (mg/L)**
Control (no irrigation)	155a	0.52a	8.96a	134a	42a	1.19a	1.14a
100%	359a	0.59a	10.41a	669a	215a	2.4b	1.88b
30%	731a	0.72a	16.97b	1512b	608b	3.61c	2.6c
LSD $_{(P=0.05)}$	0.20	0.31	0.01	0.01	0.003	0.005	0.05

Nutritional water productivity. For the micronutrients and macronutrients (calcium, copper, iron, potassium, magnesium, manganese, and zinc) examined in this study, nutritional water productivity changed considerably ($P<0.05$) between various water treatments Table 7. Interestingly, high NWP_{ca}, NWP_{cu}, NWP_{fe}, and NWP_{Mn}, NWP_{Zn} were observed under 30% ET_a relative to 100% ET_a Table 7. However, 100% ET_a high NWP_k and NWP_{Mg} Table 7. This only demonstrates that potassium and magnesium, which have a common function in the functioning and contraction of muscles, are stimulated extremely effectively with the full application of water (Mills *et al*., 2000; Maughan and Shirreffs, 2019). The results of the study concur with the observations by Sibiya (2015); Mabhaudhi *et al*. (2018), who also used similar varying water treatments in maize and sweet potato production.

Table 7: Macro and micronutrients determined on a dry mass basis of bush tea under varying water treatments (30% ET_a, 100% ET_a and control (no irrigation) of crop water requirements)

Water treatments	**NWP_Ca (mg/L)**	**NWP_Cu (mg/L)**	**NWP_Fe (mg/L)**	**NWP_K (mg/L)**	**NWP_Mg (mg/L)**	**NWP_Mn (mg/L)**	**NWP_Zn (mg/L)**
Control (no irrigation)	155a	0.52a	8.96a	134a	42a	1.19a	1.14a
100%	359a	0.59a	10.41a	669a	215a	2.4b	1.88b
30%	731a	0.72a	16.97b	1512b	608b	3.61c	2.6c
LSD (P=0.05)	0.20	0.31	0.01	0.01	0.003	0.005	0.05

NWP_ micro and macro-nutrients (ppm/mg/L): This denotes the analysis of variance concentration of micro or macro nutrients in parts per million/ milligrams per liter of water.

Multivariate data analysis

Principal component analysis (PCA) was performed for all metabolites in each varying water regime. PCA model (score) including tea tree extract showed that PC1 and PC2 explained 38.7% and 20.8% of the variance, respectively Figure 5. The PCA model showed a clear separation of the three different water states, as shown in Figure 5. This shows that there are significant differences in metabolic nutrients and their distribution in tea plant tissue in different water sources. This observation also reflects changes in the utilization of isolated metabolites (Ramabulana *et al.*, 2020). Similarly, PCA score plots were computed and validated for bush tea plant extracts under varying water regimes as indicated in Figure 5. The similarities of molecule groups were represented through score plots as shown in Figure 5 which indicated the general clustering within the datasets of bush tea plant extracts under varying water regimes.

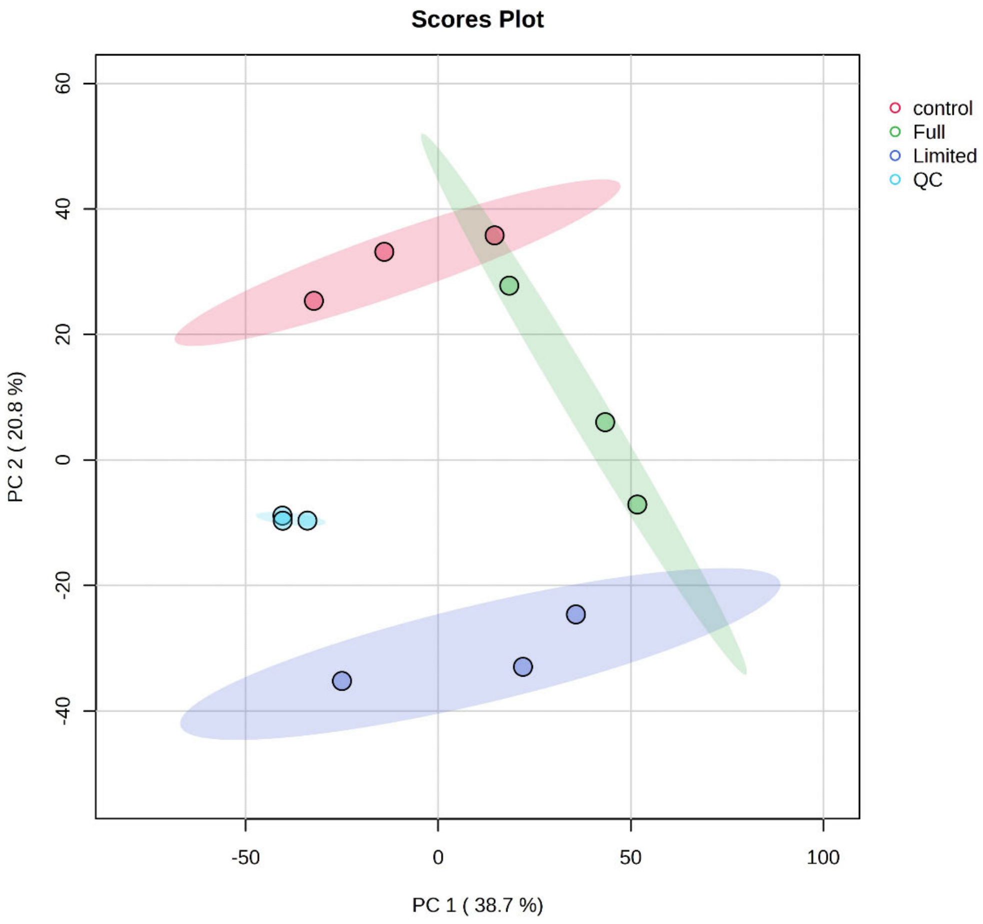

Figure 5: Multivariate data analysis of bush tea plant extracts under varying water regimes. A principal component analysis (PCA) scores scatterplot dataset obtained from LC-MS experiments with PCA 1 and PCA 2 explaining 38.7% and 20.8% of the variation indicated the general clustering within the datasets of bush tea plants of varying water regimes

Molecular network. In negative MS mode, a significant number of metabolites were found. However, mass spectra were obtained in both ESI modes (+/-). Ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS/MS) base peak intensity (BPI) chromatography of bush tea leaves under varying water regimes contained 60 compounds, all were confirmed by their retention time, mass, and molecular formula as listed in Table 8. The presence and absence of specific metabolites revealed that different water treatments led to variable regulation of secondary metabolite formation in bush tea Table 8. The identified chlorogenic acids (CGA) were found under varying water regimes with significant differences in retention time. The significant presence of chlorogenic acids (CGA) might be due to varying water regimes on bush tea plants. Our findings support prior findings by Zhang *et al*. (2018), that all herbal teas contain multiple bioactive compounds, including chlorogenic acids (CGA). Similarly, Meinhart *et al*. (2018), reported that chlorogenic acids (CGA) have various health benefits and to cure diabetes, cardiovascular disease, cancer, and obesity. This study is the first to show that different water regimes are vital for producing chlorogenic acids (CGA) in extracts of bush tea leaves.

Major chemical classes of bush tea metabolomics. Molecular networking (MN) was used to arrange MS/MS spectra into a network-shaped map based on spectral similarity, which shows structural similarity between molecules, as part of the data analysis workflow for untargeted MS/MS-based metabolomics studies (Ramabulana *et al*., 2021). The MS-Cluster algorithm was used for evaluating similarities between and within sample spectra (Frank *et al*., 2011). Ions were grouped into consensus spectra, which are represented as nodes, within a predefined mass tolerance (Frank *et al*., 2011). Based on their similarity scores (cosine scores ≥ 0.7), structurally related compounds that have comparable gas phase chemistries were classified into molecular families. In the computed molecular networking (Figure 5), 1148 consensus spectra (nodes) were generated, of which 808 were clustered and grouped into 130 independent molecular families (with a minimum of two nodes connected by an edge) using GNPS spectral matching. Self-loop nodes at the bottom of the network were used to represent spectra that were not classified into molecular families. Figure 5 shows the computed MN; 81 of the nodes were allegedly annotated through an automated library spectral matching, providing some insight into the chemical identities of the bush tea leaf extracts but also alluding to the metabolome complexity of these plants and the lack of comprehensive spectral libraries. The study of the chemical interactions between each MS/MS spectrum and the visualization of the complete metabolome found in a sample revealed structurally linked molecular families in bush tea leaf extracts under varying water regimes.

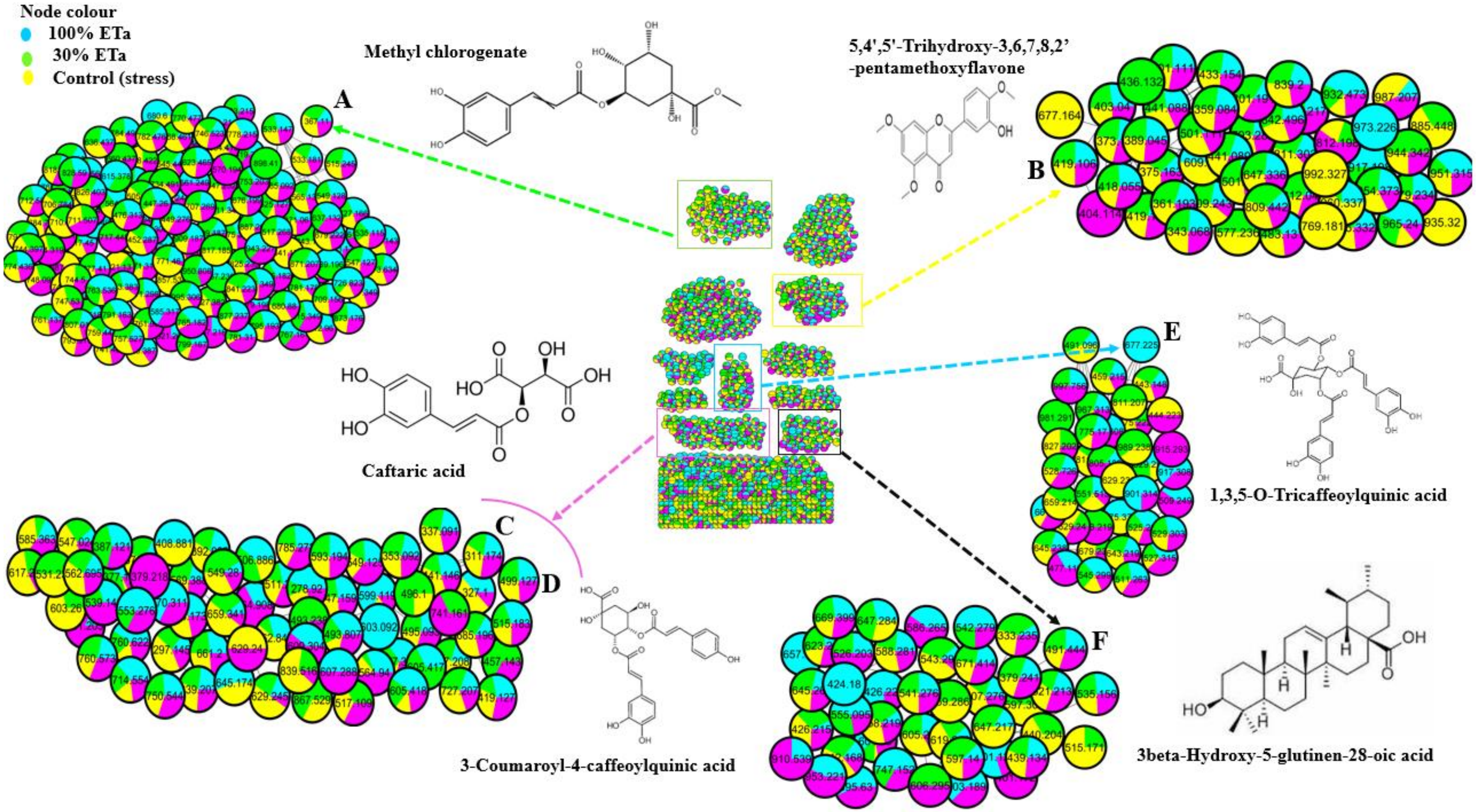

Figure 6: Molecular network of bush tea leave extracts under varying water regimes analysed by liquid chromatography–tandem mass spectrometry using electrospray ionisation in negative mode, with a molecular family (centre). Identified highlighted nodes are bioactive compounds consisting

of (**A**) methyl chlorogenate, (**B**) flavonoids, (**C**) tartaric acid, (**D** and **E**) caffeoylquinic acid, (**F**) glutinane. Node colours represent the varying water regimes of bush tea leaves extracts and the respective MS^2 spectral counts indicating the presence and absence of metabolites

Impact of water on Communic acid. Communic acid compounds are tiny, amino acids called cysteine bioactive compounds which can be obtained in several organic foods, vegetables, grains, and legumes (McIntyre *et al*., 2022). However, in herbal teas, it has not yet been identified. According to Table 8, a parent ion at m/z 301 and base peak fragments at m/z 164 and 225 are used to classify communic acid. These compounds were only detected under full water application. The results could suggest that the communic acid compound is present when the bush tea plant is irrigated fully compared to when the plant is stressed or when it is half irrigated.

Impact of water on Caftaric acid. Hydroxycinnamoyl-tartaric acid esters which have been demonstrated to have a variety of positive health impacts including antioxidant activities, are physiologically active substances (Mathatha *et al*., 2022). Based on the fragmentation patterns Rt described in Table 8 and the chemical structure as shown in Figure 6C, Caftaric acid was identified by its parent ion, $[M-H]^-$, at m/z 311. Four tartaric acids were identified in all water treatments of bush tea leave extracts. This could suggest that under different watering regimes, tartaric acid esters will still be biosynthesized in bush tea plants. These tartaric acids have not yet been identified in the leaves of bush tea.

Impact of water on Mono-Acyl Chlorogenic acids. Pei *et al*. (2016), found that the benefits of p-Coumaric acid include antioxidant, anti-inflammatory, and anti-cancer properties. The metabolites (7-11) with the molecular ion $[M-H]^-$, at m/z 337, have been determined as 5-p-Coumaroylquinic acid based on their fragmentation patterns Rt, presented in Table 8. All these compounds were identified under varying water regimes. The results could reveal that the water levels that have been used in this study integrate bioactive compounds. These findings support earlier research by Ramphinwa *et al*. (2022), who stated that bush tea contains these compounds on selected shades in relation to stress.

Impact of water on Caffeoylquinic acids. Caffeoylquinic acids are naturally occurring and are thought to be good for human health (Alcázar Magaña *et al*., 2021). Chan *et al*. (2010) and Genet (2020), reported that Caffeoylquinic acids are strong antioxidants with various beneficial effects. In this study, metabolites (12-25) were identified as caffeoylquinic acids by their parent ions (m/z 353) inferred from the fragmentation patterns Rt shown in Table 8. The caffeoylquinic acids were found to be present under all varying water regimes of bush tea leave extracts. The findings may be used to demonstrate how water use, whether limited or extensive, as well as stressful environments, affect the build-up of caffeine-quininic acids. Interestingly, our results are consistent with earlier observations by Ramphinwa *et al*. (2022), who reported that these compounds are present in bush tea plants under different shades in relation to stress.

Impact of water on Methyl chlorogenate acids. Methyl chlorogenate acids are a highly fascinating bioactive compound found in plants, with a biological function and acting as an antioxidant (Szymborska *et al*., 2022). Salem *et al*. (2022) and Zhang *et al*. (2018), reported that Methyl chlorogenate has anti-inflammatory properties; however, in bush tea, it has not been identified. Nonetheless, Methyl chlorogenate acids were identified by their parent ion, [M-H], at m/z 367 which fragmented to generate product ions at m/z 179, 119, 191, and 135 as presented in Table 8 and Figure 6A. According to the results of the current study, they are only present when the bush tea plant is irrigated with limited water (30% ET_a) because, under full application of water and water stress, these compounds were not present. This could suggest limited water as a recommended water application level in bush tea plants to synthesize methyl chlorogenate acid compounds. The current study is the first to demonstrate the geometric isomers of methyl chlorogenate acids exist on bush tea leaf extracts under varying water regimes; where the 30% ET_a had the most significant effect compared to other water regimes.

Impact of water on flavonoids. Flavonoids contain several kinds of health properties, including anti-cancer properties, anti-inflammatory, and reduced risk of chronic diseases (Gutiérrez-

Grijalva *et al*., 2017). Table 8 and Figure 6B displays the impact of water on flavonoids such as 5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone based on their parent ions at m/z 419 with the product ions at m/z 149 and 317. These compounds were detected only in 30% ET_a of bush tea leave extracts. The results could suggest that when the bush tea plant is irrigated under limited water it penetrates these compounds. Bush tea leaf extracts were found to contain 5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone molecule for the first time.

Metabolites (28) and (29) were identified as 5'-Hydroxycudraflavone A, based on the parent ions at m/z 433 based on their fragmentation patterns Rt shown in Table 8. These 5'-Hydroxycudraflavone A derivatives were served to be only present under full water application of bush tea leave extracts. The findings may simply show that these compounds won't be present in bush tea leaf extracts when there is limited water application and stress conditions.

Impact of water on triterpenoid and 3-Coumaroyl-4-caffeoylquinic acid. A pentacyclic triterpenoid was identified as the 3beta-Hydroxy-5-glutinen-28-oic acid. These compounds are derived from a natural anticancer botulin which has pharmacological properties, including cholesterol reduction (Téné *et al*., 2021). Metabolites (30-31) based on their fragmentation patterns Rt presented in Table 8, were discovered by their parent ion at m/z 491. Figure 6F shows the chemical structures in detail. In extracts of bush tea leaves with various water regimes, two of these molecules were identified. The current study's findings may indicate that different water treatments differentially regulated the formation of secondary metabolites in bush tea leaf extracts as evidenced by the presence and absence of certain metabolites. The present investigation is the first to demonstrate that bush tea leaf extracts under various water regimes include geometric isomers of 3beta-Hydroxy-5-glutinen-28-oic acid.

Metabolites (32) were annotated as 3-Coumaroyl-4-caffeoylquinic acid Table 8 and identified by a parent ion $[M-H]^-$ at m/z 499 with the chemical structure as illustrated in Figure 6D.

According to Nunes *et al*. (2021), 3-Coumaroyl-4-caffeoylquinic acid exhibit antioxidant, and it is considered a beneficial compound for human health. This compound was only observed under stress conditions. The findings could simply show that these compounds can only be detected under zero application of water because the absent of water will not limit the accumulation of this compound on bush tea plants.

Impact of water on Feruloyl-Caffeoylquinic Acids. The feruloyl-caffeoylquinic acid is a soluble phenolic compound present in many angiosperms (Dias *et al*., 2012). Metabolites (46) were identified by their parent ion at m/z 529 with fragmentation ions at m/z 179 and 173. Feruloyl-caffeoylquinic acids were detected only under limited water application. These findings may be because of the limited water that the plant has received compared to stress and full application. The current study is the first to detect feruloyl-caffeoylquinic acids on bush tea leave extracts under varying water regimes. However, it has been identified in other medicinal plants (Ramabulana *et al*., 2020).

Impact of water on Tri-Caffeoylquinic Acids. Tri-caffeoylquinic acids are specialized bioactive metabolites and are protective against biotic and abiotic stress in plants. (Uleberg *et al*., 2022). Based on their fragmentation patterns Rt, Table 8 displays the identified tri-caffeoylquinic acids with parent ions $[M-H]^-$, at m/z 677, and their chemical structures are shown in Figure 6E. On bush tea leaf extracts, these tri-caffeoylquinic acid derivatives were only found to be present when they were grown under water stress. These results might suggest that tri-caffeoylquinic acids enable bush tea plants to adapt under stress conditions with zero application of water. This study is the first to indicate the presence of tri-caffeoylquinic acids on bush tea leave extracts under varying water regimes.

Impact of water on di-caffeoylglucosides and di-caffeoyl glucarate acid. Caffeoylglucosides are a powerful antioxidant and are used to treat cancer (Ştefănescu *et al*., 2019). Based on their fragmentation patterns Rt shown in Table 8 with varied intensities, thirteen compounds containing a precursor ion, M-H]$^-$, at m/z 515 were recognized as di-caffeoylquinic acid (33-45) under all the different water regimes utilized in the investigation. The findings could indicate that bush tea accumulates these naturally occurring compounds even in the absence of water, under restricted water, and under full water application. These findings are similar to those reported by Ramphinwa *et al*. (2022), who discovered the same mass to charge 515 on bush tea plants in relation to stress. Di-caffeoyl glucarate acid (47-57), which was identified by its precursor ion [M-H]$^-$ at m/z 533 and based on its fragmentation patterns Rt shown in Table 8, was identified using a similar technique under all varying water regimes of bush tea leave extracts. A similar identification of these compounds supports Ramphinwa *et al*. (2022), who identified m/z 533 on bush tea plants to stress.

Table 8: Classification of various bioactive compounds consisting of derivatives of quinic acid (QA), flavonoids, hydroxycinnamic acid (HCA), and tartaric acid from bush tea leave extracts under varying water regimes of bush tea leaves extracts

No	**Mass to charge (m/z)**	**Retention time (mn)**	**Fragmentation ion**	**Molecular formular**	**Compound name**	**Water treatments (ET_a)**		
						30%	**100%**	**Control (no irrigation)**
1	301,2226	21,494	164, 225	C20H30O2	Communic acid		●	
2	311,039	16,745	179, 135	C13H12O9	Caftaric acid	●		
3	311,039	10,54	187, 231, 267	C13H12O9	Cis-Caftaric acid	●		
4	311,035	10,318	132, 135	C13H12O9	Caftaric acid			●
5	311,039	10,491	129, 191, 209	C13H12O9	(-)-3,5-Dicaffeoyl quinic acid	●	●	●
6	311,038	16,348	163	C13H12O9	Caftaric acid	●	●	●
7	337,0988	13,311	143,191, 209	C16H18O8	4-p-Coumaroylquinic acid		●	
8	337,0991	5,42	191	C16H18O8	5-Coumaroylquinic acid	●		
9	337,0992	2,188	153, 191	C16H18O8	5-p-trans-Coumaroylquinic acid	●		
10	337,0992	8,103	138, 153, 191	C16H18O8	5-p-Coumaroylquinic acid			●
11	353,0942	7,02	191	C16H18O9	Caffeoylquinic acid		●	
12	353,0942	5,515	191, 175, 173, 135	C16H18O9	3-O-Caffeoylquinic acid	●		
13	353,0945	7,049	353, 191, 179	C16H18O9	4-Caffeoylquinic acid	●		
14	353,0946	5,144	191,129, 209	C16H18O9	3-O-Caffeoyl-muco-quinic acid			●

15	353,0945	4,945	191	C16H18O9	trans-4-Caffeoylquinic acid	•	•	•
16	353,0946	4,747	191	C16H18O9	(+)-5-Caffeoyl quinic acid	•	•	•
17	367,08	3,694	119, 191, 135	C17H20O9	Chlorogenic acid methyl ester	•	•	•
18	419,1063	13,419	149, 317	C20H20O10	5,4',5'-Trihydroxy-3,6,7,8,2'-pentamethoxyflavone	•		
19	433,1222	14,441	133, 161, 241	C25H22O7	5'-Hydroxycudraflavone A		•	
20	491,3500	24,423	116, 299, 433	C33H48O3	3beta-Hydroxy-5-glutinen-28-oic acid		•	
21	491,3508	24,336	152, 279	C33H48O3	3beta-Hydroxy-5-glutinen-28-oic acid	•	•	•
22	499,1346	10,2	353, 337, 191, 357	C25H24O11	3-Coumaroyl-4-caffeoylquinic acid			•
23	515,1295	20,345	191, 355, 533	C25H24O12	Dicaffeoylquinic acid		•	
24	515,1290	9,781	353, 191	C25H24O12	Dicaffeoylquinic acid 1	•		
25	515,1302	9,732	353, 191	C25H24O12	Dicaffeoylquinic acid 1			•
26	515,1296	9,723	133, 161, 191, 353	C25H24O12	Dicaffeoylquinic acid 1			•
27	515,1299	9,269	191, 353, 417	C25H24O12	Dicaffeoylquinic acid	•	•	•
28	515,1295	9,381	129, 191, 357	C25H24O12	Dicaffeoylquinic acid 1	•	•	•
29	529,13	17,39	179,173	C26H26O12	Dicaffeoylquinic acid 1	•		
30	533,1042	5,033	108, 167, 191	C24H22O14	Dicaffeoylquinic acid 1		•	
31	533,1042	8,329	191	C24H22O14	Dicaffeoylquinic acid 1	•		

32	533,1045	7,051	371, 209, 173, 135	C24H22O14	Dicaffeoylquinic acid 1			•
33	533,1045	9,091	191, 375	C24H22O14	Di-caffeoyl glucarate (VII)	•	•	•
34	677,16	10,44	191, 353	C34H30O15	Tricaffeoylquinic acid 1			•
35	677,12	23,199	255, 329	C34H30O15	Tricaffeoylquinic acid 1			•

*** Shaded squares indicate the presence of metabolites under varying water regimes of bush tea leaves extracts.**

Conclusion and recommendations

Varying water regimes affect the physiological parameters of bush tea under a controlled environment. Gas exchange and chlorophyll fluorescence measurements were recorded higher in bush tea plants exposed to 30% ET_a compared to other treatments while 100% ET_a recorded a higher yield. Therefore, households in water-scarce regions may grow bush tea without compromising its nutritional benefits. However, full application yielded more compared to other water treatments. The horticultural practices of using 30% ET_a of crop water requirements caused a larger buildup of several bioactive compounds as compared to water stress and full application. Therefore, we recommend limited water application and water stress to enhance bioactive compounds in bush tea production. However, prospects led to investigations of more varying water regimes on maturity stages, nutritional water productivity, nutrient content, and phytochemical analysis on the stem and roots of bush tea extracts.

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Author Contributions

Conceptualization, **M. Rumani**, **T. Mabhaudhi** and **F.N. Mudau**; methodology, **M. Rumani**, **M.L Ramphinwa**, **A.T Ramabulana**, **N.E Madala**, **L.S Magwaza, T. Mabhaudhi** and **F.N. Mudau**.; software, **M. Rumani** and **A.T Ramabulana**; validation, **M. Rumani**, **M.L Ramphinwa**, **A.T Ramabulana**, **N.E Madala**, **L.S Magwaza**, **T. Mabhaudhi** and **F.N. Mudau**; formal analysis, **M. Rumani**, **M.L Ramphinwa**, **A.T Ramabulana**, **N.E Madala**, **L.S Magwaza**, **T. Mabhaudhi** and **F.N. Mudau**; investigation, **M. Rumani**; data curation, **M. Rumani** and **A.T Ramabulana**; writing—original draft preparation, **M. Rumani**; writing—review and editing, **M. Rumani**, **M.L Ramphinwa**, **A.T Ramabulana**, **N.E Madala**, **L.S Magwaza, T. Mabhaudhi** and **F.N. Mudau**; supervision**, N.E Madala**, **L.S Magwaza**, **T. Mabhaudhi** and **F.N. Mudau**; funding acquisition, **T. Mabhaudhi** and **F.N Mudau**. All authors have read and agreed to the published version of the manuscript.

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CHAPTER FOUR

INVESTIGATING THE INFLUENCE OF VARYING WATER REGIMES ON THE GROWTH AND DEVELOPMENT, NUTRITIONAL WATER PRODUCTIVITY AND QUALITY OF BUSH TEA (*ATHRIXIA PHYLICOIDES* DC.).

Muneiwa Rumani[1*], Tafadzwanashe Mabhaudhi[2,3], Takudzwa Mandizvo[1], Anza-Tshilidzi Ramabulana[4], Ntakadzeni Edwin Madala[4], Maanea Lonia Ramphinwa [5], Lembe Samukelo Magwaza[1,6], Fhatuwani Nixwell Mudau[1*]

[1]School of Agricultural Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa
[2]Centre of Transformative Agricultural and Food Systems, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa
[3]International Water Management Institute (IWMI-SA), South Africa, Pretoria, South Africa
[4]Department of Nutrition, Faculty of Health Sciences, University of Venda, Private Bag X5050, Thohoyandou, Limpopo 0950, South Africa
[5]Department of Plant and Soil Science, Faculty of Sciences, Agriculture and Engineering, University of Venda, Private Bag X5050, Thohoyandou, Limpopo 0950, South Africa
[6]Department of Food Systems and Development, Faculty of Natural and Agricultural Sciences, University of Free-State, PO. BOX 330, Bloemfontein 9300, Republic of South Africa

*Corresponding author: muneiwaabigail@gmail.com; mudauf@ukzn.ac.za

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Abstract. Bush tea (*Athrixia phylicoides* DC.) is an indigenous shrub of South Africa known for its medicinal properties and bioactive compounds. However, due to a lack of comprehensive information to develop suitable irrigation management strategies, the cultivation of bush tea is currently limited to small-scale operations. In light of this, the research investigated the effect of water regimes on the growth and development, yield, nutrient content, metabolite composition and along with its nutritional water productivity (NWP) under field conditions. The treatment consisted of a crop water requirement (ET_a) of 100%, 30% and the control (stress) in a Complete Randomised Block Design (CRBD), each treatment was replicated three times to ensure robustness and accuracy of the findings. Bush tea plant extracts were meticulously analysed using Ultra-High-Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS) to comprehensively assess the metabolite composition of bush tea. Results demonstrated that the highest gravimetric moisture content readings were in the control treatment and the lowest in the 100% treatment. Conversely, the 30% ET_a treatment significantly ($P<0.05$) impacted the growth, development, productivity, NWP and quality of bush tea. However, it was observed that the 100% ET_a treatment resulted in a higher biomass yield (259.1 Kg. ha $^{-1}$) compared to the 30% ET_a treatment (171.2 Kg. ha $^{-1}$) and control (stress) (68.2 Kg. ha $^{-1}$). Additionally, the Water Productivity (WP) exhibited notable differences across the varying water regimes. The results demonstrated that bush tea leaf extracts are abundant in phytochemically diverse metabolites each playing a distinct physiological role, thus exhibiting differential distribution patterns influenced by the varying water applications. Notably, our results elucidated the presence of various metabolite classes, such as terpenoids, chlorogenic acids, flavonoids and lipids influenced by varying water regimes. This study marks the first comprehensive decoding of the bush tea metabolome under varying water regimes.

Keywords: herbal tea, water productivity, physiological parameters, molecular network, phytochemicals

Introduction

Bush tea (*Athrixia phylicoides* DC.) is a perennial, aromatic, indigenous shrub cultivated in South Africa, well-regarded for its traditional use as an herbal tea and medicinal herb (Lerotholi et al., 2017). The plant is known to possess pharmacological properties, and its leaves and stems are rich in bioactive compounds that hold significant medicinal value, including the treatment of heart disease, headaches, and infected wounds (Mudau et al., 2007; Lobo et al., 2010). Additionally, its compounds have been associated with potential benefits in managing high blood pressure, infected throats, and blood purification (Rakuambo et al., 2009). Notably, the edible parts of the plant contain notable levels of polyphenols, tannins, antioxidants, quercetin, and flavonoids, contributing to its therapeutic significance (Tshivhandekano et al., 2018). Despite its valuable attributes, bush tea plants are still primarily gathered from the wild, prompting efforts for their domestication and commercialization (Reichelt et al., 2012; Lerotholi et al., 2017). Current research has emphasized the influence of cultural practices, mineral nutrition (Mudau et al., 2007; Tshivhandekano et al., 2018), pruning (Mohale et al., 2018), and environmental conditions (Tshivhandekano et al., 2013; Ramphinwa et al.; 2022) on the growth, development, and quality of the plant.

Notably, while bush tea has exhibited some water stress tolerance compared to other tea cultivars, investigations into the impact of varying water regimes on its production and quality remain limited (Ponmurugan et al., 2016). Water is considered a crucial resource promoting vegetative growth and reproductive stages, particularly in arid and semi-arid regions, where fluctuations in rainfall can significantly affect plant growth and quality (Rostamza et al., 2011; Chivenge et al., 2015). Prior research has highlighted the influence of water stress on various plant species, indicating its significance in plant biomass, leaf area, and secondary metabolite production (Chiappero et al., 2019; Gatabazi et al., 2019). Given the importance of water in bush tea cultivation, an investigation into the impact of varying water regimes on its growth, development, yield, and quality is imperative. Therefore, the primary objective of this study was to assess the response of bush tea to different water regimes, particularly focusing on plant growth, development, yield, and quality.

Material and Methods

Plant material, site description and experimental design

A field experiment was conducted from spring to summer season in the year 2022 at the Controlled Environment Research Unit (CERU), located at the University of KwaZulu-Natal (UKZN) in Pietermaritzburg, South Africa (29˚37'S; 30˚16'E). The region experiences an annual rainfall of 400mm, with 45% occurring during rainy days from June to August, typical of its humid, sub-tropical climate. Temperature fluctuations are significant, ranging from a maximum of approximately +/- 33 ˚C during summer to a minimum of around +/- 26 ˚C in winter, as detailed in Table 9. The experimental soil was randomly sampled at the beginning of the trial and taken to the Institute for Commercial Forestry Research (ICFR) Analytical and Research Laboratory to determine chemical and physical properties. The type of soil at the experimental site is predominantly characterized as loam Table 9. The bush tea stem cuttings used in the study were sourced from the Agricultural Research Council (ARC) in Nelspruit, Mpumalanga province, South Africa (25.4518˚ S, 30.9697˚ E). Bush tea plants were transplanted to the experimental field 80 days after the cuttings were set (Maedza et al., 2015).

Table 9: Physical and chemical characteristics of soil in the field

Clay (%)	**Organic C (%)**	**pH (KCl)**	**P (cmol/kg)**	**Ca (cmol/kg)**	**K (cmol/kg)**	**Mg (cmol/kg)**	**Na (cmol/kg)**	**N (%)**
21.1	2.70	5.20	15.75	8.16	0.68	1.49	0.05	0.19

The experimental design was laid out in a Complete Randomised Block Design (CRBD) with three treatments (100%, 30%, and control (stress) of crop water requirement (ET_a)), replicated three times. The total plot area was 100 m^2, the plant population was kept at 6944.44 plants/ha, The size of the individual plots was 10 m × 10 m. Each plot consisted of two plant rows, 1.2 m, and 0.75 m (inter- and intra-row spacing), giving a total of 12 plants per plot. Weeding was conducted manually before crop establishment and thereafter weekly until the crop reached full maturity.

The irrigation schedule was determined by the daily crop water requirements from the product of normal tea (unshaded) crop factors (K_c), as published by Singh (2013) Table 10, and the monthly average reference evapotranspiration (ET_o) was determined on a daily basis throughout the experimental period, enabling an accurate timely response to the plant's water needs. The on-site automatic weather station of the Discipline of Agrometeorology was used to collect the evapotranspiration reference data. All treatments were irrigated to reach field capacity before the commencement of the treatments. This practice was adopted to minimize potential losses from evaporation and drainage and to ensure that the soil maintained adequate moisture levels during the critical peak periods of the day. As a result, the crop water requirement ET_a was calculated using Eq. 1:

$$ET_a = ET_o \times K_c \qquad (1)$$

Table 10: Crop water requirements of bush tea in response to varying water regimes

		ET_o	**ET_a**	**Duration**	**Total water applied**
	K_c	mm	mm	days	mm
Initial	0.95	10.2	9.96	40	408
Mid-season	1.00	8.61	8.61	61	525.21
Late season	1.00	12.2	12.2	28	341.60
Water applied (ET_a)					
• **100%**					1274.81
• **30%**					382.44
• **0%**					0

K_c=crop factor based on Allen et al., (1998), ET_o = reference evapotranspiration, ET_a = crop water requirement which was measured during the spring to summer season of the year 2022.

Data collection

Weather data was obtained from an automated weather station (AWS) using honest observer by onset (HOBO) data logger sensors (Onset Computer Corporation, USA) Table 11. The data was measured during spring and summer cropping season, from 30th August to 1st November 2022. Daily meteorological data considered included minimum (T_{min}) and maximum (T_{max}), air temperature (˚C), solar radiation, relative humidity rainfall and reference evapotranspiration (mm) was calculated using the Food Agriculture Organization (FAO) calculator. The soil water content was determined through gravimetric sampling. Six soil samples were collected at a depth of 30 cm from all water treatments in each plot and sealed in zip-lock bags to prevent moisture loss. Subsequently, the gravimetric water content using Eq. 2:

$$(\boldsymbol{\theta g}) = \left(\frac{\boldsymbol{\theta}\mathbf{wet}-\boldsymbol{\theta}\mathbf{dry}}{\boldsymbol{\theta}\mathbf{dry}}\right) \times \mathbf{100}\ \% \qquad (2)$$

where:

Ɵg = Gravimetric moisture content (%),

ƟWet = wet soil (g) and

ƟDry = dry soil (g).

Eq. 3 was used to calculate volumetric water content from gravimetric water content.:

$$(\boldsymbol{\theta v}) = \boldsymbol{\theta g} \times \left(\frac{\boldsymbol{Psoil}}{\boldsymbol{Pwater}}\right) \tag{3}$$

where:

Ɵv = Volumetric moisture content (%),

Ɵg = Gravimetric moisture content (%),

Psoil = the bulk density of that given soil (g/cm^3) and

Pwater = water density (g/cm^3).

Crop water use was then determined using Eq. 4:

$$\boldsymbol{ET_a} = \boldsymbol{P} \pm \Delta\boldsymbol{SWC} \tag{4}$$

where:

ET_a = actual evapotranspiration (mm),

P = Precipitation (mm) and

ΔSWC = changes in soil water content (mm)

Table 11: Rainfall and temperature data for the duration of the experimental period from the UKZN CERU field

	Total	**June**	**July**	**August**	**September**	**October**	**November**
Rainfall mm	299	11	9	30	42	95	110
Temp$_{min}$ (°C)	90	10	9	30	11	13	16
Temp$_{max}$ (°C)	150	22	24	26	28	23	25

Temp$_{min}$ and Temp$_{max}$ represent the minimum and maximum temperatures, respectively, recorded during the growing season.

Morphological measurements

Leaf gas exchange and chlorophyll measurements were measured using LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) integrated with an infrared gas analyser (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). Measurements were taken on the third half-fully formed leaf from the plant’s tip between 08.30 and 11.30 a.m. by clamping the leaf inside the sensor head, with analysis conducted from the vegetative stage to harvesting, with three plants from each treatment, replicated three times.

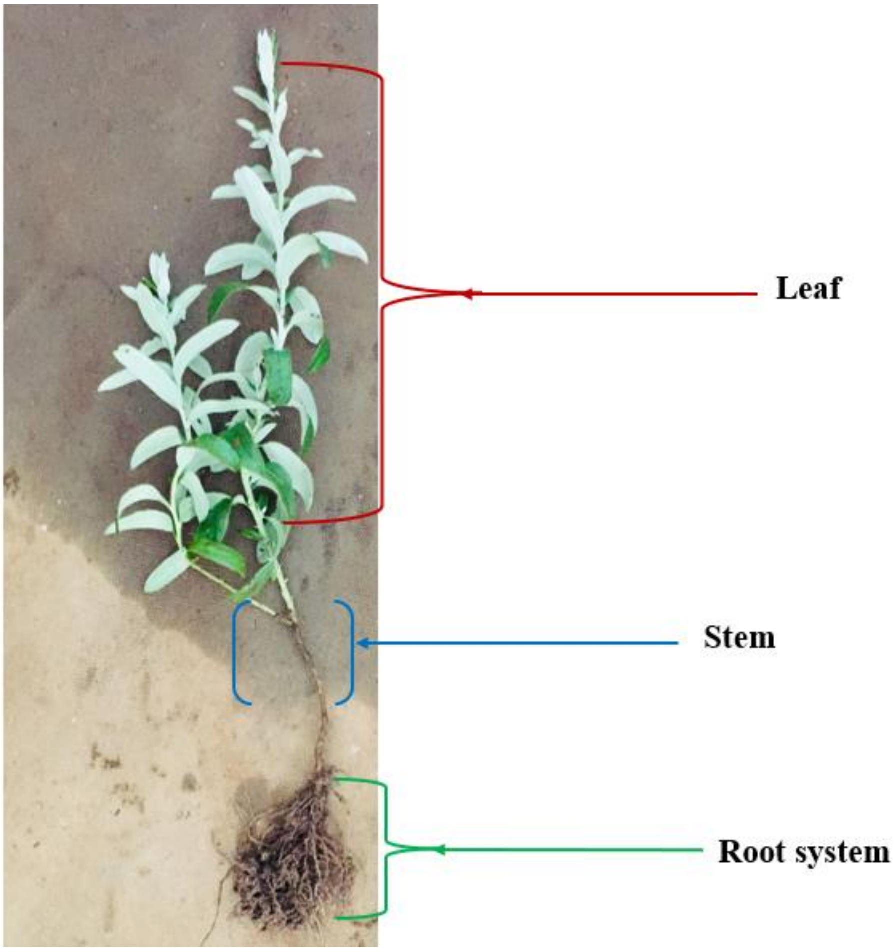

Figure 7: Bush tea plant at harvest

Yield.

The assessment of yield included the measurement of biomass, with the collection of bush tea samples taking place 119 days after planting. A comprehensive assessment of bush tea yield components was conducted by sampling three plants for each treatment. The measurements recorded on a plot basis included the yield, focusing on the fresh above-ground mass.

Determination of water productivity

Water productivity was calculated using Eq. 5:

$$WP = Y_a / ET_a \tag{5}$$

where: WP is water productivity (kg/m^3), Ya is the biomass and ET_a is the water applied based on crop water requirement.

Determination of nutritional composition

The ariel parts of bush tea plant was freeze-dried using a model RV3 vacuum freeze drier (Edwards, United States of America) immediately after yield determination to preserve nutrients and avoid further metabolic reactions. Thereafter, samples were ground using mortar and pestle and analysed for nutritional content. The nutrients analysed per dry matter basis included micro-nutrients (copper, zinc, iron, magnesium, manganese, and sodium) and macro-nutrients (calcium and potassium).

Determination of nutritional water productivity (NWP)

Nutritional water productivity was calculated using Eq. 6:

$$NWP = (Y_a / ET_a) \times NC \tag{6}$$

where: NWP is the nutritional water productivity (nutrition m^{-3} of water evapotranspired), Ya is the actual harvested yield (kg/ha), ET_a is the water applied based on crop water requirement (m^3/ha), and NC is the nutritional content (kg^{-1}).

Metabolites extraction

The extraction of metabolites followed a protocol adapted from Makita et al. (2016). Initially, freeze-dried bush tea leaves were finely powdered, and 2 g of the resulting powder was mixed with 20 mL (1:10 m/v) of 80% aqueous methanol (Romil SpS, Cambridge, UK). Subsequently, the homogenate was subjected to overnight spinning in a digital rotisserie tube rotator at 70 rpm, followed by centrifugation at 5000 g for 20 minutes to remove debris. The resulting supernatant was carefully transferred to fresh, clean tubes. To obtain the final extracts, a 1:1 (v:v) dilution in methanol with 500 µL inserts was performed, achieving a final volume of 20 mL. The extracts were then filtered through 0.22 µm nylon filters into cylindrical quartz glass vials (2 x 10 cm) and a 2-mL vial fitted with a 0.2-mL conical bottom glass insert (Ramabulana et al., 2020). Three (3) independent replications for each sample group were prepared; thereafter, placed in a column oven set at 40 ˚C. To evaluate the quality of the generated data, quality control (QC) samples were generated by pooling equal quantities of each sample and running them simultaneously with experimental samples.

Liquid Chromatography-Quadruple Time-of-Flight Tandem Mass Spectrometry (LC-MS/MS)

The mass spectrometry (MS) instrument (LCMS-9030 QTOF, Shimadzu Corporation, Kyoto, Japan) was utilized for the analysis. At 55 ˚C, a Shim-pack Velox C18 column (100 mm x 2.1 mm, with a particle size of 2.7 m) was used for the chromatographic separation on bush tea extracts. A binary mobile phase gradient comprising of solvent A: 0.1% formic acid in Milli-Q water (both HPLC grade, Merck, Darmstadt, Germany) and solvent B: methanol (UHPLC grade, Romil SpS, Cambridge, UK) with 0.1% formic acid and injection volume of 3 L was used for all samples (Makita et al., 2016; Augustin et al., 2011). The high-definition mass spectrometer QTOF was used to further analyse on the chromatographic effluents to collect information on negative electrospray ionization. The following settings were made: 4.0 kV for the interface voltage, 300 ˚C for the interface temperature, 3 L/min for nebulization and dry gas flow, 400 ˚C for the heat block, 280 ˚C for the DL, 1.8 kV for the detector voltage, and 42 ˚C for the flight tube (Ramabulana et al., 2020). High mass accuracy was monitored using sodium iodide (NaI) as a calibration solution to obtain typical mass accuracies with a mass error below 1 ppm, and a range of *m/z* 100 to 1000 above an intensity threshold of 5000, MS1 and MS2 were sequentially created (via data dependent collection). Argon was used as the collision gas for the MS/MS fragmentation experiments along with MS^E mode, and the spread of the collision energy was 12 eV to 25 eV (Ramabulana et al., 2020). Pooled samples were used for quality control (QC), LC-MS system conditioning, and non-linear signal correction. Thus, the QC samples were injected at the start and end of the batch. Sample acquisition was also randomised to track and rectify variations in the instrument response.

Molecular networking and metabolite annotation

The online workflow (http://gnps.ucsd.edu/) was used to build molecular networks on the GNPS website (accessed in February 2023). The Shimadzu LCMS-9030 QTOF's raw data was transformed into an open-source format (mzML) before being uploaded to the online workflow (Aron et al., 2020). Any MS2 fragment ions within +/- 17 Da of the precursor m/z were removed from the data after it had been submitted through filtering. The MS/MS spectra were window filtered by selecting only the top 6 fragment ions within the +/- 50 Da window across the spectrum. The data were then clustered using the MSCLUSTER technique i.e., Global Natural Products Social Molecular Networking (GNPS) (Aron et al., 2020). The precursor ion and the MS2 fragment ion were set to a mass tolerance of 0.05 Da (Aron et al., 2020). A minimal cosine score of 0.7 was created with more than six matched peaks (interconnections between metabolites/similarity). Only nodes in each other's respective top 10 comparable nodes were preserved in the network. The highest-scoring edges were eliminated, and a limit of 100 nodes may be joined into a single molecular family. The library spectra were filtered in the same way as the sample spectra. Cytoscape software was used to visualize the produced molecular networks (https://gnps-cytoscape.ucsd.edu/process?task=767b2f3fe4754e0a9c67e5b4917355b4) (Shannon et al., 2003). Using their empirical formulas, which were created utilizing precise mass and fragmentation patterns discovered during MS2 data, all matched and some unmatched nodes were validated or putatively annotated. Also, they were evaluated against various well-known natural product dereplication databases like; KNApSAcK Core System (http://www.knapsackfamily.com/knapsack_core/top.php), PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://www.chemspider.com/) (Aron et al., 2020). Metabolomics Standards Initiative (MSI) was used for metabolite annotation. The produced network that was produced was subsequently investigated using network annotation propagation (NAP), which involved structural searches in the database, including GNPS. In GNPS, substructure annotation was performed using the MS2LDA interface.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® version 20 (VSN International, UK). The least significance difference (LSD) was used to separate means at the 5% significance level.

Results and Discussion

Soil water content

The data in Figure 8 displays the gravimetric sampling of soil water content (SWC) under different water regimes (ET_a levels) over a period of five weeks. The results illustrate significant differences ($P < 0.05$) in soil moisture levels among the water treatments. Notably, the 30% ET_a water treatment exhibited the highest SWC, peaking at 15% during the study period, followed by the 100% ET_a treatment with a maximum of 12%, and the control group with a peak SWC of 9%. These findings underscore the critical role of soil water content in influencing various aspects of plant development, productivity, and soil temperature, as well as the overall water dynamics within the soil (Deutsch et al., 2010). Moreover, the presence of higher moisture content in the unsaturated root zone indicates that bush tea plants prefer moderately moist soil conditions rather than excessively wet environments (Choi and Jacobs, 2007). These observations may be attributed to the complex interplay of factors such as soil texture and pore size distribution, which impact water depletion rates in different plant species (Gatabazi et al., 2022).

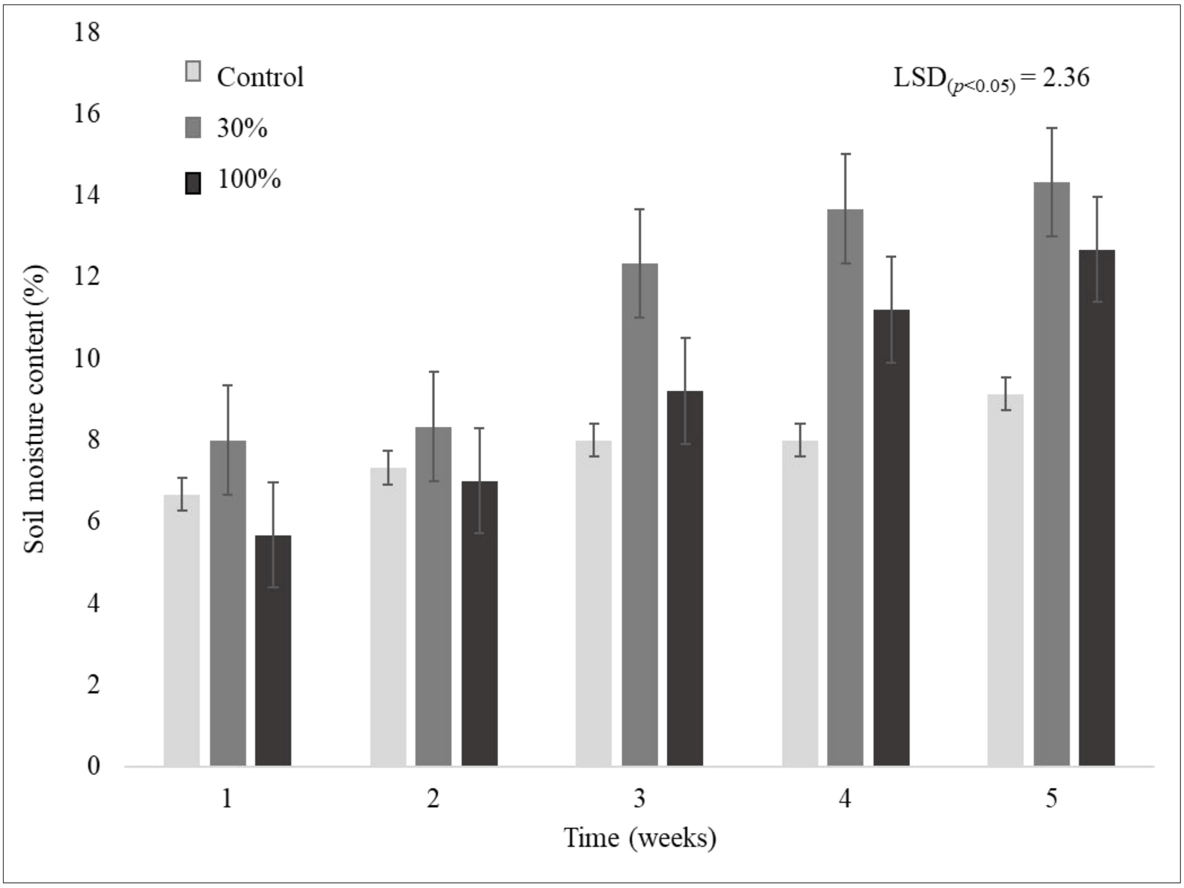

Figure 8: Soil water content (%) under varying water regimes of bush tea over five-week period

Yield

The study revealed significant differences (P < 0.05) in yield parameters, specifically in yield and water productivity, among varying water treatments for bush tea plants (Table 4.4). The highest recorded yield was observed in the 100% water treatment, amounting to 259.1 kg.ha^{-1}, surpassing the 30% treatment, which yielded 171.2 kg.ha^{-1}. Conversely, the control treatment exhibited the lowest yield at 68.2 kg.ha^{-1}. This reduction in yield under the control treatment could be attributed to water stress, a known factor limiting plant growth and crop yield across various agricultural settings (Gatabazi et al., 2015). These findings align with the observations made by Keshavarz-Mirzamohammadi et al. (2021), who noted that water stress during the vegetative phase can impede cell division and expansion, ultimately leading to reduced plant growth and development.

Additionally, water-deficient plants tend to close stomata and restrict nutrient uptake in the root system, culminating in decreased dry matter accumulation (Keshavarz & Khodabin, 2019). Furthermore, drought-induced reductions in the leaf area index (LAI) during the growing season can limit biological yield by diminishing photosynthetic rates (Yang et al., 2015). In terms of water productivity, water treatments also exerted a significant influence ($P < 0.05$). The 100% water treatment exhibited the highest water productivity, with a yield of 570.5 kg.m^3, outperforming other treatments Table 12. These results are consistent with those presented by Rahil (2022), who emphasized the direct relationship between the volume of water applied and the observed results. This outcome was likely influenced by the full application of water in the 100% treatment. The current study's results are in line with those reported by Mabhaudhi et al. (2019) and Motsa et al. (2015), who employed similar water treatments in their research on sweet potato production.

Table 12: Yield components of bush tea under varying water treatments (30% ET_a, 100% ET_a and control)

Water treatments (ET_a)	**Yield (kg/ha)**	**WP (kg/m^3)**
Control	68.2a	0a
30%	171.2b	570.5b
100%	259.1c	259.1c
LSD$_{(p < 5\%)}$	7.91	8.33

Water productivity (WP) represents the total biomass, including leaves. The yield and WP values are statistically significant according to the LSD (5% level).

Nutritional composition

The results indicated that the nutritional composition of bush tea plants was significantly influenced by the varying water regimes ($P < 0.05$). Notably, the 30% ET_a treatment demonstrated the highest nutritional composition, with increased levels of essential elements such as Calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and zinc (Zn) compared to the 100% ET_a and water stress treatments Table 13. The observed lower nutritional composition under water stress conditions could be attributed to the adverse effects of drought on nutrient uptake in plants, resulting in altered ion concentrations within plant tissues (Corell et al., 2012). These findings are consistent with the research of Gunes et al. (2006), who similarly reported decreased nutrient uptake and mineral nutrient concentrations in crops under drought stress conditions.

Table 13: Nutritional composition of bush tea under three water treatments (30% ET_a, 100% ET_a and control)

Water treatments (ET_a)	**Ca (mg/L)**	**Cu (mg/L)**	**Fe (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**Mn (mg/L)**	**Zn (mg/L)**
Control	127.3a	0.207a	9.60a	137a	43.4a	2.133a	2.173a
30%	624.7c	2.433c	18.07c	1175.3c	817.0c	10.20c	7.933c
100%	422.0b	1.523b	13.60b	870.0b	501.0b	4.803b	3.40b
LSD$_{(p<0.05))}$	4.5	0.23	2.3	1.78	1.73	1.60	6.90

Nutritional water productivity

The results obtained from the study demonstrate a significant difference ($P < 0.05$) in the micronutrient parameters, including Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), and Zinc (Zn), under varying water treatments Table 14. Notably, the NWP_Ca (78694 m^{-3}) was found to be higher under the 30% ET_a water treatment compared to the other treatments. Similarly, the NWP_K (1175.3 m^{-3}) was observed to be higher under the 30% ET_a treatment in comparison to the 100% ET_a (870.0 m^{-3}) and stress conditions (0 mg/L). The interaction between NWP and micronutrients and macronutrients to water treatments was also found to be significantly different ($P < 0.01$). These results can be attributed to the varying responses of plants to water stress, influenced by the intensity and duration of the stress (Hosseinzadeh et al., 2018). Additionally, the findings align with previous observations on yield, water productivity (WP), and nutritional content (Mabhaudhi et al., 2019), highlighting the potential of bush tea to provide nutrition even under limited water conditions.

Table 14: Nutritional water productivity of bush tea under three water treatments (30% ET_a, 100% ET_a and control)

Water treatments	**NWP_Ca (mg/L)**	**NWP_Cu (mg/L)**	**NWP_Fe (mg/L)**	**NWP_K (mg/L)**	**NWP_Mg (mg/L)**	**NWP_Mn (mg/L)**	**NWP_Zn (mg/L)**
Control	0a	0a	0a	0a	0a	0a	0a
30%	78694c	317.9c	18.07c	1175.3c	817.0c	10.2c	1018.7c
100%	17296b	62.8b	13.60b	870.0b	501.0b	4.80b	789.7b

LSD **($p<0.05$)**	5.68	6.01	2.0	1.78	1.63	1.60	6.90

NWP_ micro and macro-nutrients (ppm/mg/L): This denotes the analysis of variance concentration of micro or macro nutrients in milligrams per liter of water.

Leaf gas exchange and chlorophyll fluorescence measurements

The findings from this study reveal a significant effect of varying water regimes on the physiological parameters, including leaf gas exchange and chlorophyll fluorescence in bush tea Table 15 and 16. Photosynthetic rates were significantly influenced by varying water regimes, showing distinct differences among the treatments. Notably, the 30% ET_a treatment exhibited the highest rates, with A net photosynthetic rate values of 29.57 and 36.18 µmol m^{-2} s^{-1} CO_2 during weeks 1 and 2, respectively. Additionally, the 100% ET_a treatment displayed improved photosynthetic rates, recording values of 22.79 and 26.80. In contrast, the control treatment exhibited the lowest photosynthetic rates, with values of 17.64 and 24.19 µmol m^{-2} s^{-1} CO_2 for weeks 1 and 2, respectively. These findings suggest that the 30% ET_a treatment provided the most conducive conditions for photosynthetic rate in bush tea, resulting in significantly higher rates compared to the control and 100% ET_a treatments. The results emphasize the plant ability to efficiently utilize water, even under limited water conditions, thereby enhancing photosynthetic activity and fostering sustained growth and productivity.

Notably, the control treatment displayed significantly lower stomatal conductance (0.11 and 0.37 µmol m^{-2} s^{-1} CO_2) compared to the other water treatments. This reduction in stomatal conductance is often associated with water stress, which affects various physiological and metabolic processes, leading to decreased gas exchange and inhibited photosynthesis (Shao et al., 2008). These results align with previous observations on Citron melon production under stress conditions (Mandizvo et al., 2022), emphasizing the impact of reduced stomatal conductance and transpiration rate on water conservation and plant physiological functioning (Mandizvo et al., 2021). Moreover, the control (stress) treatment exhibited high water use efficiency, indicating that it efficiently utilized water through stress mechanisms such as reduced gas diffusion and transpiration rate. The 30% ET_a treatment displayed the highest water use efficiency indices (WUEi) of 38.25 and WUEinst of 113.4 compared to the 100% ET_a treatment with WUEi of 34.19 and WUEinst of 107.2. This suggests that the 30% ET_a treatment effectively maintained tissue water status, minimized water loss, and produced stable yields, highlighting the potential of bush tea crops to thrive even with limited rainfall. Furthermore, chlorophyll fluorescence measurements indicated a higher proportion of photosynthetic rate under the 30% ET_a treatment compared to other water treatments, which could be attributed to the relatively lower water received by the bush tea plants under stress conditions and full irrigation. These findings further support the idea that bush tea can thrive well under limited water conditions, even in water-scarce regions, as demonstrated in provinces like KwaZulu-Natal (Mudau et al., 2015).

Table 15: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence measurements of bush tea under three water treatments (30% ET_a, 100% ET_a and control)

Leaf gas exchange measurements

Source of variance	**d.f**	***gs***	***T***	***A***	***Ci***	***A/Ci***	***Ci/Ca***	***Wu_i***	***WUE_{inst}***
Water treatment	2	0.0672744**	99.730**	236.4336**	444986.0**	1.37390**	86.49**	54.6706**	512.482**
Time	1	0.1734605**	614.302*	1679.5086**	628587.7**	103.43761**	789.04**	1232.8113**	14192.359**
Water treatment x time	2	0.0056122*	32.338*	70.7924**	24220.3**	1.25047**	75.24**	5.4087**	222.444**
Residual	12	0.0005902	2.505	0.4742	828.5	0.03739	12.44	0.9491	3.111

Leaf gas exchange measurements

Source of variance	**d.f**	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***
Water treatment	2	212670.4**	264909**	0.0364162**	39.9027**	6.9390**	0.85000**	3487.627**
Time	1	395624.4**	2004*	0.0799728*	1120.0131**	1.6563**	1.21607**	11604621**
Water treatment x time	2	55781.1*	584*	0.0142603*	3.4191**	0.1244**	0.84353**	1722146**

Residual	12	739.4	9033	0.0006282	0.3031	0.2717	0.01148	16521

d.f; degrees of freedom, **g_s**; stomatal conductance, **T**; transpiration rate, **A**; net CO_2 assimilation rate, **A/C_i** ; CO_2 assimilation rate/intercellular CO_2 concentration, **C_i**; intercellular CO_2 concentration, **C_i/C_a**; ratio of intercellular and atmospheric CO_2, **WUE_i**; intrinsic water use efficiency, **WUE_{ins}**; instantaneous water-use efficiency, **F_v/F_m**; maximum quantum efficiency of photosystem II photochemistry, **Φ_{PSII}**; the effective quantum efficiency of PSII photochemistry, **qP**; photochemical quenching, **qN**; non-photochemical quenching, **ETR**; electron transport rate, **ETR/A**; relative measure of electron transport to oxygen molecules, ***** and ****** denote significant at 5 and 1% probability levels, respectively, **Ns**; non-significant.

Table 16: Means of leaf gas exchange and chlorophyll fluorescence measurements of bush tea under three water treatments (30% ET_a, 100% ET_a and control)

Chlorophyll fluorescence measurements

Week 1	ET_a	***gs***	***T***	***A***	C_i	A/C_i	C_i/C_a	***WUEi***	WUE_{inst}	***Fo***	***Fm***	***Fv/Fm***	Φ_{PSII}	***qP***	***qN***	***ETR***	***ETR/A***
	Control	0.11a	29.27a	17.64a	229.31a	1.73a	0.27a	17.47a	41.94a	2573.0a	2233a	0.05a	15.59a	0.03a	0.95a	533a	30.43a
	30%	0.38c	33.31c	29.57c	276.02c	3.43c	0.87c	21.63c	48.09c	2769.8c	2669c	0.30c	19.20c	2.20b	2.37a	981c	27.11c
	100%	0.27b	30.74b	22.79b	254.31b	1.93b	0.71b	20.04b	46.31b	2641.2b	2444b	0.16b	17.86b	1.23ab	1.23a	849b	37.30b

Chlorophyll fluorescence measurements

Week 2	ET_a	***gs***	***T***	***A***	C_i	A/C_i	C_i/C_a	***WUEi***	WUE_{inst}	***Fo***	***Fm***	***Fv/Fm***	Φ_{PSII}	***qP***	***qN***	***ETR***	***ETR/A***
	Control	0.37a	35.86a	24.19a	462.82a	7.06a	8.50a	32.35a	84.33a	2055.50a	2264a	0.22a	29.69a	0.83a	0.99a	1050a	25.48a
	30%	0.53c	47.69b	36.18c	757.11c	7.17a	22.22c	38.25c	113.45c	2607.52c	2667b	0.78c	36.24c	2.94b	1.00b	3640c	76.49c
	100%	0.45b	45.59b	26.80b	661.03b	7.25a	10.85b	34.19b	107.21b	2431.40b	2478ab	0.35b	34.04b	1.50ab	0.99a	2491b	54.40b

d.f; degrees of freedom, $\mathbf{g_s}$; stomatal conductance, **T**; transpiration rate, **A**; net CO_2 assimilation rate, (μmol CO_2 m^{-2} s^{-1}), $\mathbf{A/C_i}$; CO_2 assimilation rate/intercellular CO_2 concentration, $\mathbf{C_i}$; intercellular CO_2 concentration, $\mathbf{C_i/C_a}$; ratio of intercellular and atmospheric CO_2, $\mathbf{WUE_i}$; intrinsic water use efficiency, $\mathbf{WUE_{ins}}$; instantaneous water-use efficiency, $\mathbf{F_v/F_m}$; maximum quantum efficiency of photosystem II photochemistry, $\mathbf{\Phi_{PSII}}$; the effective quantum efficiency of PSII photochemistry, **qP**; photochemical quenching, **qN**; non-photochemical quenching, **ETR**; electron transport rate, **ETR/A**; relative measure of electron transport to oxygen molecules, **AES**; alternative electron sinks. Varying upper-case letters within a column indicates significant differences among water treatments. $\mathbf{g_s}$; (mmol m^{-2} s^{-1}), **T**; (mmol H2O m^{-2} s^{-1}), **A**; (μmol CO2 m^{-2} s^{-1}**)**, $\mathbf{A/C_i}$; (μmol. mol m^{-1}), $\mathbf{C_i}$; (μmol. mol m^{-1}), $\mathbf{WUE_i}$; (μmol (CO2) m^{-2}); $\mathbf{WUE_{ins}}$, (μmol. mol^{-1}), Fv/Fm; (ratio); **ΦPSII**, the effective quantum efficiency of PSII photochemistry; $\mathbf{q_P}$, photochemical quenching; $\mathbf{q_N}$, non-photochemical quenching; **ETR**, (μmol e^{-1} m^{-2} s^{-1}); **ETR/A**, (μmol e $μmol^{-1}$ CO2).

Molecular networking

A computational approach tool called; molecular network (MN) classifies metabolites based on structural similarity with compounds that share similar structural moieties and are grouped together to form a molecular family. The data were obtained in both (+/-) electrospray ionisation (ESI); however, most metabolites were found to ionize better in the negative MS mode. Thus, the data obtained was used to identify metabolites. When the GNPS site generates metabolites with similar fragmentation patterns, a molecular network is used to interpret and visualize the complex data that results from the MS data Figure 9. All the MS/MS spectra in the dataset are compared for possible similarities, which allows the molecular network to propagate annotation to unknown but related compounds (Wang et al., 2016). Thus, the results show the known and unknown compounds with similar fragmentations which share common structural features Figure 9. Chlorogenic acids such as the 1-Caffeoyl-4-deoxyquinic acid, caffeoylquinic acid, and Alpha-D-glucosyl-(1->3)-beta-D-mannose Figure 9, are biologically active compounds which are shown to have various health benefits. Some of these compounds have been identified in leaves of bush tea leaves, and the results show fragmentation changes in responses to water treatments.

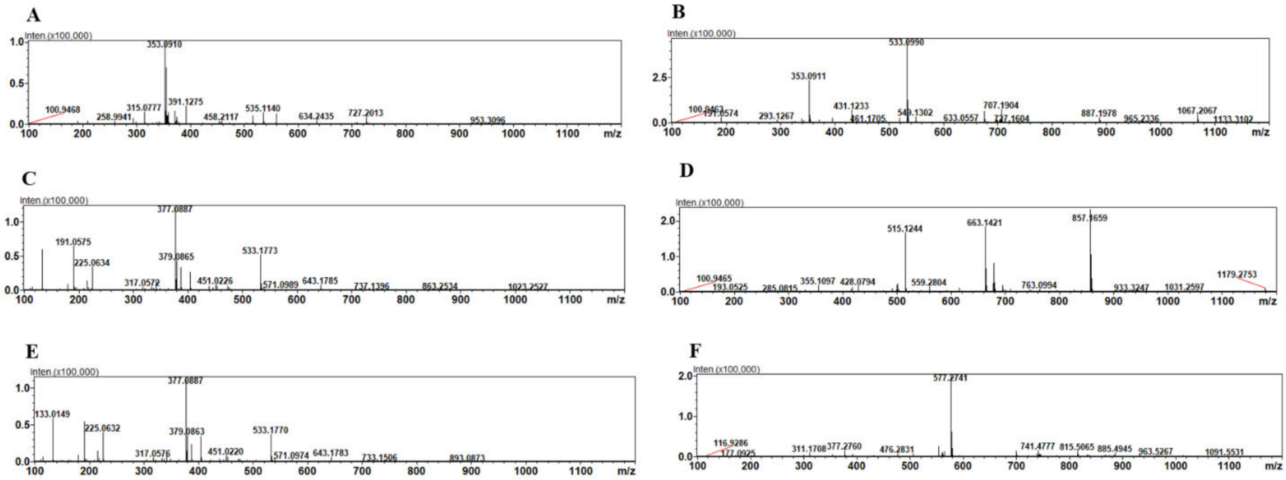

Figure 9: Typical mass spectra of the fragmentation patterns of (A) Caffeoylquinic acid (B), Luteolin 7-O-(6"-malonylglucoside) (C), Quinic acid (D), Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose (E) 1-Caffeoyl-4-deoxyquinic acid (F) Crenulatoside A (-) as listed in Table 17

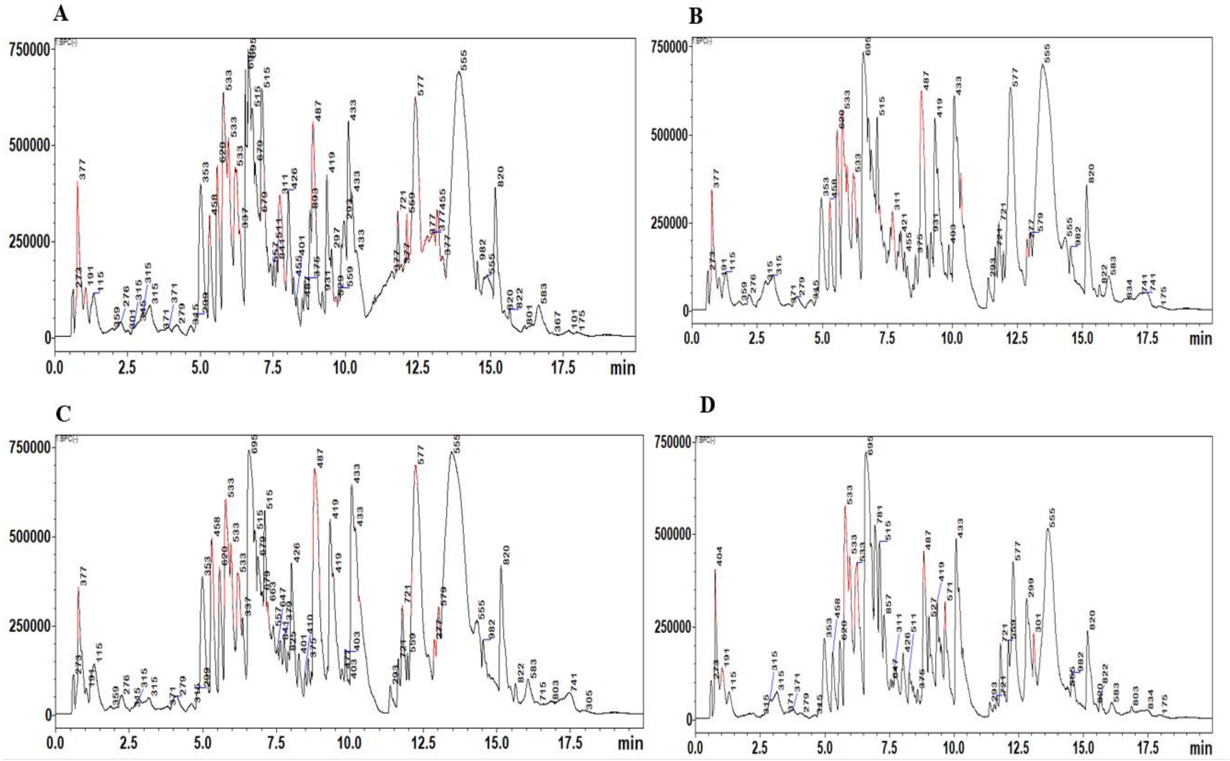

Figure 10: Comparison of UHPLC-QTOF-MS profile chromatograms in responses to varying water regimes (A: 100%, B: 30%, C: Control of crop requirements, D: QC (Quality control)) of bush tea leaves samples using a biphenyl column

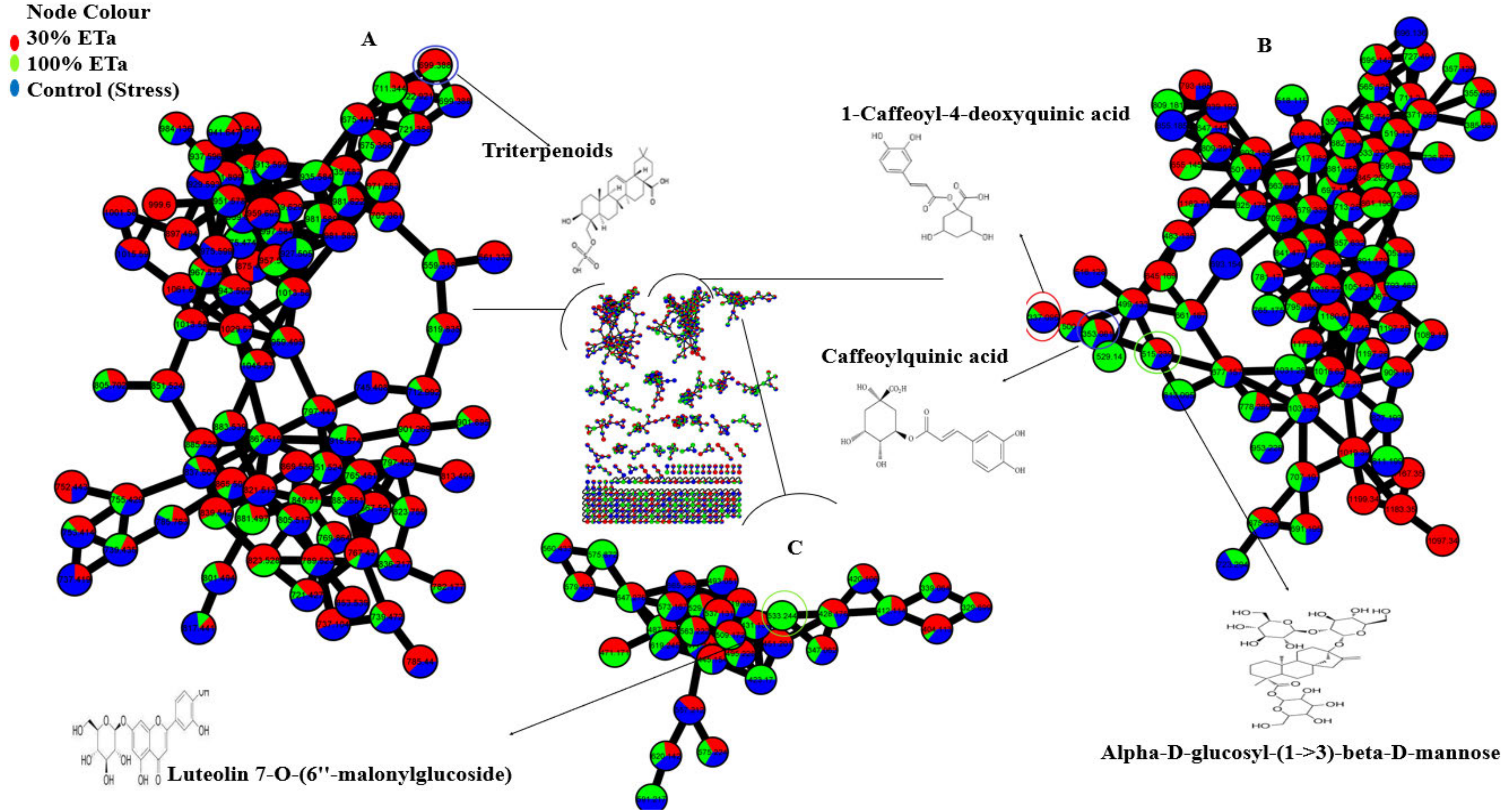

Figure 11: Molecular network of the three water regimes of bush tea leaves extracts analysed by liquid chromatography–tandem mass spectrometry using electrospray ionisation in negative mode, with a molecular family (centre) indicating five major metabolite classes identified, highlighted

nodes are: triterpenoids (**A**), flavonoids (**B**), chlorogenic acid (**C**). Highlighted nodes are also the aglycones/backbones from which the flavonoids identified are derived. Node colours represent the varying water regimes of bush tea leaves extracts and the respective MS^2 spectral counts indicating the presence and absence of metabolites

Major chemical classes of bush tea metabolomes

In classical molecular networking, the MS-Cluster algorithm is utilized to compare the similarity of ions and cluster them into consensus spectra, which are then represented as nodes. With the aid of similarity scores (cosine scores 0.7), metabolites sharing similar gas-phase chemistry and related structural properties were organized into molecular families. Through GNPS spectral matching, we generated 644 consensus spectra (nodes) in the computed molecular network (MN), with 463 of these nodes clustered into 181 independent molecular families, each composed of a minimum of two edge-connected nodes. Self-ringing nodes were employed at the bottom of the grid to depict spectra that were not assimilated into any molecular families Figure 11. Within the computed MN Figure 11, 60 of the nodes were tentatively annotated through automated spectral matching of the library, unveiling the chemical identities of the three bush tea water regimes. Additionally, this process emphasized the complexity of the metabolome within these plants and highlighted the scarcity of comprehensive spectral libraries. By scrutinizing the chemical relationships among each MS/MS spectrum and visualizing the distinct metabolites found in bush tea leaf extracts under varying water regimes, we were able to discern structurally related molecular families.

A high number of lipids, including triterpenoids Figure 11A, were found under plants subjected to 30% and 100% ET_a of crop water requirements of bush tea leaves and were grouped into a molecular family Figure 11. Triterpenoids are widely distributed in both edible plants and epiphytes (Augustin et al., 2011). Triterpenoid metabolism plays an important role in the development of a medicinal plant, and it represents the largest group of phytochemicals (Haung et al., 2022). Moreover, it is considered the most abundant secondary metabolite (Dinda et al., 2010). These are high molecular weight, structurally complex tri-silicon molecules, linked to one or more sugar molecules through glycosidic linkage (Jouaneh et al., 2022; Ramos et al., 2022). In the current study, triterpenoids identified are a class of terpenes with 30 carbons Ramos et al. (2022) and are divided into two main classes: tetracyclic and pentacyclic compounds (Jouaneh et al., 2022; Kareru et al., 2008; Huang et al., 2022). Therefore, the results set a new perspective that adequately demonstrates that plants subjected to 30% and 100% ET_a of crop water requirements in bush tea result in the accumulation of triterpenoids due to the application of water at different levels. The results show significant differences amongst the varying water regimes because this compound was present under full and limited water applications besides under zero application of water.

Further, this is due to enzymes involved in glucose metabolism when the plant accumulates water to prevent the development of insulin resistance and normalize plasma glucose and insulin levels (Jouaneh et al., 2022). Moreover, it is also because these compounds increase resistance to physical and psychological stress (Kareru et al., 2008). As shown in Figure 11A, plants subjected to 30% and 100% ET_a of crop water requirements produced distinct triterpenoids that were clustered in their molecular family. This suggests that the application of water at different water levels of bush tea leaves' chemically related metabolites are distributed differently according to the molecular family Figure 11. The findings indicate that these compounds may be found on bush tea leaves with the presence of water, yet under stress cannot be found.

Other metabolites that are widely distributed under varying water treatments of bush tea leaves are flavonoids, which are clustered into a molecular family Figure 11B. Flavonoids are commonly present in fruits and vegetables and have been linked to several of health benefits, including antioxidant, anti-cancer, and anti-bacterial activity (Samanta et al., 2011; Panche et al., 2016). They are used by plants for growth and, among other things, protection against oxidative stress (Samanta et al., 2011; Xue et al., 2021). Despite conserving flavonoid production in plants, reductase activity, isomers, dioxygenases, and hydroxylases cause several subclasses to emerge (Treutter, 2006; Haralampidis et al., 2002). Moreover, they are divided into six (6) major subclasses according to their chemical structure (flavones, isoflavones, flavanones, flavonols, flavan-3 oles (flavonols), anthocyanins (Ghosh, 2020). Flavonoids contain structurally diverse sand components as backbones, namely chalcones, flavones, isoflavones, flavanols, flavonols, flavanones, and anthocyanidins (Agati et al., 2020; Rahimi et al., 2019; Ghosh, 2020). These backbones occur in different modified forms of through hydroxylation, methylation, and glycosylation via transylases (Agati et al., 2020). Here, it was noted that the plants subjected to 30% ET_a of crop water requirements were abundant in flavonoids and polyphenols in structure and biosynthesized through the phenylpropanoid pathway. As a result, flavonoid (Luteolin 7-O-(6"-malonylglucoside) were found under plants subjected to 30% ET_a of crop water requirement Figure 11B. According to the findings, bush tea is more likely to have an active Luteolin 7-O-(6"-malonylglucoside) compound when it is watered with a limited amount of water as compared to when it is applied fully or under stress (zero application of water).

Figure 11C displays the chlorogenic acids such as (1-Caffeoyl-4-deoxyquinic acid, caffeoylquinic acid, and alpha-D-glucosyl-(1->3)-beta-D-mannose) based on their parent ions at m/z 337, 353, and 515 under plants subjected to three water levels (30% ET_a, 100% ET_a, and control of crop water requirements) Figure 11C. Which cluster into a molecular family Figure 11C that helped in a putative annotation. Chlorogenic acids are a class of phenolic compounds widely distributed in various plant sources such as fruits and vegetables, coffee beans, tea, apple, and wine (Kim and Park, 2019). They are described as effective in preventing weight gain, inhibiting the development of liver steatosis, and blocking insulin resistance (Naveed et al., 2018). It is one of the most abundant beneficiary polyphenols in plants well known as nutritional antioxidants in plant-based foods (Liang and Kitts, 2015). Plants produce structurally diverse phenolic compounds which play a significant photo-protective role (Liang and Kitts, 2015). These include various subgroups (caffeoylquinic, p-coumaroylquinic and feruloyquinic acids) and classes of organic compounds (quinic acids and derivatives) (Kim and Park, 2019; Abbas-Mohammadi et al., 2022). Three water treatments were highlighted as being rich in chlorogenic acids. In the current study, it was observed that chlorogenic acids were present in all these water treatments, which presented similar pseudo-molecular ions with the same fragmentation patterns (Raheem et al., 2019). The results could simply be intended to show that chlorogenic acids compounds were observed under various water treatments of bush tea leaves, this simply shows that whether there is an application of water or not chlorogenic acids can be present on bush tea leaves.

The number system used below refers to the sequence of the presentation Table 17 where metabolites are listed according to increasing m/z vales showing significant differences in responses to water treatments. As well as the fragmentation patterns over time.

Effects of water treatments on chlorogenic acid

Three (3) mono-Acyl Chlorogenic Acids (CGAs) were observed with parent ions, $[M-H]^-$ at m/z 191. Metabolites (34-35) were annotated as quinic acid based on their parent mass and detected molecular formula Table 17. Derivatives of monoacyl chlorogenic acids (CGAs) were observed only in bush tea leaves under plants subjected to 30% ET_a of crop water requirements. The results showed that this molecule was detected under limited water because it was not detected under full application (100% ET_a) of water nor under stress conditions (control). It has been speculated that CGA could play critical roles (antioxidant, antiviral, antibacterial, anticancer) in the regulation of lipid and glucose metabolism, and thus aid in a wide range of potential health benefits (Murugesu et al., 2017; Raudone et al., 2022). Results show that human consumption of bush tea may protect against cardiovascular disease and some types of cancer due to this compound. The results also imply that in agricultural practices, using limited water to save water in a water-scarce area is recommended, because using limited water will not limit the chlorogenic acids compounds in bush tea leaves.

Effects of water treatments on mono-caffeoyl quinic acid

In this study, ten (10) mono-caffeoyl quinic acids were identified as shown in Table 17 and which produced a molecular ion, $[M-H]^-$, at m/z 353, were identified and annotated as caffeoylquinic acids (CQAs) (1-10). These molecules have been detected in large amounts in bush tea leaves from different water regimes. Altogether, the results of the current study together with those published elsewhere Clifford et al., (2006) and Ramabulana et al. (2020), show that geometrical isomerization of metabolites is a plausible, non-enzymatic approach used by plants to maximize the metabolite formation and can serve multiple purposes as shown below. All water treatments responded significantly at m/z 353. Ramphinwa et al. (2022), observed similar results, who also detected m/z of 353 on bush tea plants for the first time. However, mono-caffeoyl quinic acid was detected under all water treatments of bush tea leaves, these are because it is a type of polyphenol, a class of micronutrients known for their antioxidant properties. This is related to the health benefits that are mainly attributed to the chlorogenic acids found in medicinal plants. On the other hand, this compound has pharmacological effects for the treatment of diabetes and the common cold (Clifford et al., 2006). The interest in these molecules lies in their health benefits. The results intend to reveal whether bush tea plants are irrigated with full application, limited water or zero water application, mono-caffeoyl quinic acid can still be observed.

Effects of water treatments on di-caffeoylquinic acid (Di-CQA)

A similar approach was taken in the identification of di-caffeoylquinic acids (Di-CQA) (18-24) identified by the precursor ion $[M-H]^-$ at m/z 515 and based on the fragmentation patterns and Rt shown in Table 17. Despite the difference in density, these particles were identified in all water treatments of bush tea leaves. Thus, seven (7) di-caffeoylquinic acid (Di-CQA) in Table 17 was found to be present in bush tea leaves. These are bioactive compounds that have been shown to have various health benefits and antioxidant and anti-inflammatory properties (Moyo et al., 2022). Similarly, Di-CQA derivative has been shown to display antioxidant activity and various other health benefits Clifford et al., (2020); Ramabulana et al., 2020). Our findings showed strong evidence for the presence of Di-CQA on bush tea leaves under different water regimes. On the other hand, Bush tea leaves were synthesized under all water levels, as evidenced by the results, which demonstrate that varying water application levels activate these compounds Table 17. The results show significant responses of water treatment to Di-CQA which shows the changes in fragmentation under varying water regimes. The results simply recommend that even if we don't irrigate, we can still find these compounds in bush tea leaves.

Effects of water treatments on di-caffeoyl glucarate acid

In this study, eight (8) di-caffeoyl glucarate acid were identified which have a precursor ion, $[M-H]^-$, at m/z 533 as shown in Table 17. This compound may occur as a metabolite from soluble conjugated with organic acids and /sugars (Ramabulana et al., 2020). As shown in Table 17, metabolites (25-32) were annotated as luteolin 7-O-(6"-malonylglucoside) based on their molecular formula. These di-caffeoyl glucarate acid derivatives have been identified in leaves of bush tea under limited water application and stress conditions. The results simply demonstrate that bush tea does not manufacture these compounds when it receives more water. Thus, this uniqueness could be due to the biosynthesis of these compounds in the aryl parts of the leaves caused by different applications of water (Farzaei et al., 2016). Di-caffeoyl glucarate acid's presence contributes to the medicinal plants' health benefits (Tajner-Czopek et al., 2020). Notwithstanding, it is also a newly formed dicaffeoyl glucaric acid derivative that contributes to the desired taste, flavor, and aroma (Farzaei et al., 2016). Therefore, the m/z was affected by water treatments and simply means that water treatments influence the fragmentation of di-caffeoyl glucarate acid in responses to varying water regimes. This simply brings the knowledge to people in Sub-Saharan Africa (SSA) to use limited water when irrigating bush tea plants, because limited water does not compromise di-caffeoyl glucarate acid compound.

Effects of water treatments on p-Coumaroyl-Caffeoylquinic Acids (pCo-CQAs)

According to the authors `information, p-Coumaroyl-Caffeoylquinic Acids (pCo-CQAs) have not been identified in bush tea leaves. Four (4) of these compounds were observed (14-17) with an intense product ion at m/z 337. Four (4) of these compounds were observed under all water treatments of bush tea leaves. These simply aim to detect the presence of 1-Caffeoyl-4-deoxyquinic acid which is intensely stronger because it has been detected under varying water regimes. Mostly, under irrigated bush tea. It has been proven that p-Coumaroyl-caffeoylquinic acids are bioactive food compounds that exhibit several important therapeutic properties (Bazyiko et al., 2015; Ruiz et al., 2013). The findings of the current investigation demonstrate the changes in responses to water treatments on bush tea leaves. The results also show changes in fragmentation which utilises or synthesize these compounds with or without the use of water. In terms of the agricultural practices, bush tea can be grown in water-scarce areas and can still generate p-Coumaroyl-Caffeoylquinic Acids.

Effects of water treatments on feruloyl-Caffeoylquinic Acids (F-CQAs)

Table 17 displays the characterization of Feruloyl-Caffeoylquinic Acids (F-CQAs) based on their parent ions at m/z 599 and 529. Previous research by Liu et al. (2020) and Clifford et al., (2020), has associated Alpha-leusteric acid with various health benefits and its role in chronic human diseases. Metabolites 11 and 36 were identified as Crenulatoside A (-). Interestingly, these compounds were only detected when water use was limited to plants subjected to 30% ET_a. Alcázar Magaña et al., 2021, have reported that caffeine derivatives, including these compounds, possess potent antioxidant properties and offer several health benefits. Additionally, these compounds can serve as raw materials for promoting health benefits (Dall’Acqua et al., 2008). The results indicate that when water use is restricted, the activity of F-CQAs on bush tea increases compared to full water usage and stressful conditions. This can be attributed to the abundance of bioavailable and bioactive polyphenols present in bush tea. Furthermore, the study identified three alpha-Eleostearic acid compounds (37-39) with a precursor ion, [M-H]-, at 277 Table 17. Notably, alpha-Eleostearic acid was detected under both plants subjected to 30% and 100% ET_a treatments, while it was not present under the stress condition. This suggests that the plant is unable to synthesize this compound in the absence of water. Moreover, the results emphasize the significant role of water in plants, as the compound is undetectable without water application.

Table 17: Tartaric acid from tissues (leaves) of bush tea receiving varying water regimes

No	**Mass to charge (m/z)**	**Retention time (mn)**	**Molecular formular**	**Fragment ion**	**Compound name**	**Water treatments (ET_a)**		
						30%	100%	Control (stress)
1	353,0909	4,98	C16H18O9	191, 179, 135	Caffeoylquinic acid	✓		
2	353,0907	5,178	C16H18O9	191,179,173,135	Caffeoylquinic acid	✓		
3	353,091	5,817	C16H18O9	191,179,173,136	Caffeoylquinic acid	✓		
4	353,0911	5,0111	C16H18O9	191,179,135	Caffeoylquinic acid		✓	
5	353,0908	4,944	C16H18O9	191,179,135	Caffeoylquinic acid		✓	
6	353,0909	5,899	C16H18O9	191,179	Caffeoylquinic acid			✓
7	353,0909	6,255	C16H18O9	191,179,173,135	Caffeoylquinic acid			✓
8	353,0909	5,0885	C16H18O9	191,179,173,135	Caffeoylquinic acid	✓		✓
9	353,0907	5,92	C16H18O9	191,179,173,135	Caffeoylquinic acid	✓		✓
10	353,091	5,962	C16H18O9	191,179,173,135	Caffeoylquinic acid	✓		✓
11	599,325	3,904	C29H44O5	82,138,135	Tritepenoids	✓		
12	377,0891	0,849	C18H18O9	353, 335, 191, 173, 135	1-Caffeoyl-4-deoxyquinic acid	✓		
13	377,088	0,763	C18H18O9	191	1-Caffeoyl-4-deoxyquinic acid	✓		
14	337,0957	6,279	C16H18O8	191	1-Caffeoyl-4-deoxyquinic acid	✓		
15	337,0959	6,356	C16H18O8	191	1-Caffeoyl-4-deoxyquinic acid		✓	
16	337,0958	6,361	C16H18O8	191	1-Caffeoyl-4-deoxyquinic acid		✓	
17	337,0958	6,281	C16H18O8	191	1-Caffeoyl-4-deoxyquinic acid			✓
18	515,1247	6,796	C18H28O17	353, 335, 191, 173, 135	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose	✓		
19	515,1246	6,771	C18H28O17	353, 335, 191, 173, 135	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose	✓		

20	515,1246	6,746	$C_{18}H_{28}O_{17}$	353, 335, 191, 173, 135	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose		✓	
21	515,1248	6,761	$C_{18}H_{28}O_{17}$	353, 335, 191, 173, 135	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose		✓	
22	515,1251	7,144	$C_{18}H_{28}O_{17}$	353, 335, 191, 173, 136	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose			✓
23	515,1248	6,832	$C_{18}H_{28}O_{17}$	353, 335, 191, 173, 135	Alpha-D-galactopyranuronosyl-(1->2)-6-deoxy-L-mannose			✓
24	515,12443	7,08	$C_{18}H_{28}O_{17}$	353, 335, 191, 173, 135	Alpha-D-glucosyl-(1->3)-beta-D-mannose	✓		✓
25	533,177	0,911	$C_{30}H_{30}O_{9}$	287,151,135	Bismurrangatin		✓	
26	533,0989	6,021	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)		✓	
27	533,177	0,934	$C_{30}H_{30}O_{9}$	287,151,135	Bismurrangatin			✓
28	533,0988	5,857	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)			✓
29	533,0988	5,2	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)	✓		✓
30	533,1042	6,07	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)	✓		✓
31	533,099	6,025	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)	✓		✓
32	533,0909	5,962	$C_{24}H_{22}O_{14}$	287,151,135	Luteolin 7-O-(6"-malonylglucoside)	✓		✓
33	191,0211	1,058	$C_{6}H_{8}O_{7}$	155, 111	Quinic acid	✓		
34	191,021	1,031	$C_{6}\ H_{8}\ O_{7}$	155,111	Citric acid	✓		
35	191,0574	0,826	$C_{7}H_{12}O_{6}$	155,111	Quinic acid			✓
36	529,1363	9,095	$C_{27}H_{46}O_{10}$	367,335,193,173	Crenulatoside A (-)	✓		
37	277,219	12,955	$C_{18}H_{30}O_{2}$	185,251	Alpha-Eleostearic acid	✓		
38	277,2195	12,975	$C_{18}H_{30}O_{2}$	185,251	Alpha-Eleostearic acid		✓	

39	277,195	13,036	C18H30O2	185,251	Alpha-Eleostearic acid	✓

***Shaded tick indicates the presence of metabolites under varying water regimes of bush tea leaves extracts**

Conclusion and Recommendations

The findings of this study demonstrate the substantial impact of different water regimes on the growth and yield of bush tea plants. Notably, the highest yield was observed in plants subjected to 100% ET_a, followed by those under 30% ET_a and water stress treatments. Interestingly, the 30% ET_a treatment significantly improved soil water content, nutritional composition, and nutritional water productivity, emphasizing the importance of optimizing water application in bush tea cultivation. Control (stress) conditions led to decreased stomatal conductance but increased water use efficiency, suggesting the adaptability of bush tea to water-scarce environments. Consequently, varying water regimes emerge as a crucial factor in influencing plant physiology, nutritional composition, leaf gas exchange, and chlorophyll fluorescence. Moreover, the impact of the 30% ET_a treatment on the plant's chemistry resulted in the formation of novel metabolites not typically found in the plant. Similarly, the control (stress) treatment exhibited a significant accumulation of newly formed metabolites compared to the 100% ET_a treatment. This underscores the potential of the plant to adapt and synthesize unique compounds under stress conditions, making it a valuable resource for food and nutritional security in arid regions. The current investigation highlights the use of metabolomics in conjunction with agronomic practices, specifically varied water regimes, as a promising avenue to enhance the metabolite composition of bush tea leaves. The chemotaxonomic diversity revealed through unique saponin presence under different water treatments suggests the potential for novel natural product discovery. Further exploration of the molecular network and computational tools in the GNPS system would shed light on the specific metabolites influenced by varying water treatments, facilitating the identification of potential new bioactive compounds. This approach may extend to the analysis of stem and root metabolomes to uncover additional chemical constituents. Future studies should encompass an in-depth analysis of nutritional water productivity, water use efficiency, and molecular networking of bush tea, including its stem and roots, under diverse water regimes, especially in contrasting environmental conditions. These investigations will provide a comprehensive understanding of the plant's adaptive mechanisms and the potential bioactive compounds it may offer, contributing to the advancement of sustainable agriculture practices and natural product research.

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Author Contributions

M. Rumani, M.L. Ramphinwa, A.T. Ramabulana; designing of the experiment, data acquisition, analysis, data interpretation and drafting of the article. **F.N. Mudau, T. Mabhaudhi, N.E. Madala**: Conceptualization, supervision, methodology, revision, and approval of submission of article. **T. Mandizvo, L.S Magwaza, F.N Mudau:** Formal analysis, Writing original draft

Conflict of Interest

The authors declare no conflicts of interest.

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

The research background revolves around investigating the potential of varying water regimes to enhance the growth and development as well as the quality of bush tea while also facilitating its production for medicinal purposes. However, the scarcity of water resources has exerted a significant adverse impact on contemporary global concerns, particularly in Africa, as the region's population continues to grow (Kotir, 2011; Chappell and LaValle, 2011). Significant issues caused by population growth and climate change have increased pressure on food security, especially affecting countries with water problems such as South Africa (Kotir, 2011). Although the South African state is committed to ensuring food security, food insecurity continues to increase in the country (de Cock *et al*., 2013; Chakona and Shackleton, 2019). As the problem of food security increases with diminishing resources and population growth (Chakraborty, 2011), small-scale agriculture is also increasing to meet the increasing demand (Lenkabula, 2006; Makunga *et al*., 2008).

Research on bush tea is considered a solution to the problem of malnutrition in agriculture (Chivenge *et al*., 2015; Mudau *et al*., 2022). This approach promises to improve current food practices. In countries facing water scarcity and malnutrition as studied by Bain *et al*. (2013), bush tea cultivation turns out to be a beautiful plant with the potential to create new markets for the rural economy and contribute to the conservation of plant biodiversity and the search for plant-derived compounds for new medicines. It can also be a way to generate income for rural communities. The production of bush tea in SA is limited due to its growth, especially in tropical regions where water and soil fertility are inadequate (Mudau *et al*., 2022). However, it must be acknowledged that the availability of water and nutrients may cause problems in achieving good growth and good characteristics in bush tea.

This study provides evidence of improved bush tea yield, NWP and nutrient yield, and nutrient water efficiency in water-limited regions. The main idea of this study is that the growth, production, growth, yield and nutritional content of bush tea are not affected by different water sources. To investigate this proposal, we conducted two experiments at the UKZN in the year 2022, one in a controlled environment and the other in a field environment.

The initial experiment, detailed in **Chapter three**, was designed to assess the responses of physiological parameters, nutritional water productivity, and comprehensive quality analysis, including the examination of bush tea metabolites under different water regimes. Subsequently, the second experiment, presented in **Chapter four**, aimed to assess plant growth and development, nutritional water productivity, and phytochemical analysis under varying water conditions for bush tea. The outcomes from both experiments consistently demonstrated that limited water application at 30% ET_a outperformed full water application at 100% ET_a and the control, which received no water (under water stress conditions). Nevertheless, it is noteworthy that the 100% ET_a treatment still yielded satisfactory results, likely due to the substantial water input compared to the other water application levels. Furthermore, despite exhibiting reduced photosynthetic efficiency under water stress, bush tea is likely attributable to its natural habitat in arid environments (Mudau *et al*., 2022). As a result, the initial hypothesis was ultimately rejected.

5.2 Challenges

- The preliminary trials failed because it was conducted during the winter season; however, the trial was then set up in the spring and summer season, and it was successful.
- Transplanting shock occurs in winter; however, this experiment was conducted from spring to summer season.
- Aphids, termites, and mealy bugs attacked the bush tea plant species, in both the controlled tunnel facility and the field experiments. Nevertheless, their population was effectively managed through weekly applications of insecticides.

5.3 Recommendations

The following recommendations are based on the findings made from the investigation:

- These findings offer valuable insights for future research, suggesting the exploration of diverse environments and seasons, both within and outside the province, for cultivating bush tea. This approach could lead to improvements in its nutritional water productivity and overall yield.

- It would be interesting to investigate the interaction of bush tea with other herbal medicinal teas in future studies.
- Future studies should investigate the water utilization and the water productivity of different medicinal plants, since this study only focused on bush tea, both in a controlled environment and in a field experiment.
- To achieve a comprehensive understanding, future studies should consider the exploration of additional experimental variables, such as agronomic management practices like planting density. This will help in the scientific and systematic evaluation of their impact on the yield and quality of many bush tea varieties.
- Quantitative research of bush tea harvested under varying water regimes by means of comparative assessments through questionnaires in different locations to provide quality assessments.
- Molecular network on the stem and roots of bush tea is highly recommended, in order to give more insight into the compounds available in bush tea plants under varying water treatments and in different locations and seasons.

The Literature Review (**Chapter Two**) showed that bush tea is one of the herbal teas with a significant number of nutrients (Olivier *et al*., 2012), which makes it a unique multi-purpose plant (Rakuambo, 2007). Its medicinal purposes make it an important plant to be maintained during its growth stages, to enhance its production (Lerotholi *et al*., 2017). This study therefore emphasized the measurements relating to its physiological (growth, development, and productivity), as well as its Nutritional Water Productivity (NWP), yield, and quality. **Chapter two** provides a comprehensive overview of the insights offered by numerous researchers concerning herbal teas, with a primary focus on bush tea, to show the importance of cultivating bush tea under diverse conditions to stimulate its growth and enhance its quality within agricultural production. It also contributes to a deeper understanding of its water utilization, yield potential, and nutritional attributes. The cultivation of bush tea holds substantial promise for improving food production across much of sub-Saharan Africa while preserving its nutrient-rich composition.

Chapter three assessed the responses concerning the growth, development, productivity, yield, nutritional water productivity, and quality attributes of bush tea. This evaluation involved the utilization of varying water regimes within a controlled environmental facility. Findings show that water scarcity and water stress have an impact on plant growth, yield, and quality. In particular, bush tea growth and productivity were significantly affected when compared to the optimal water conditions within a controlled environment. These results are also reflected in the growth and development pattern of the tea plant, which is more effective in unrestricted water, especially at 30% ET_a, compared to other water treatments. An additional noteworthy observation about the enhanced nutritional water productivity observed in bush tea under conditions of restricted water application, specifically at the 30% ET_a of crop water requirements. Drip irrigation effectively reduced soil evaporation losses due to its ability to get a smaller surface area. Considering that bush tea typically exhibits shallow root systems, adopting a regular irrigation schedule that ensures continuous moistening of the root zone can offer advantages. Consequently, the crop remained capable of accessing water within its root zone even under conditions of limited water availability. The investigation yielded evidence suggesting that the implementation of drip irrigation methods could potentially enhance crop yields. Furthermore, the results indicated that a higher concentration of bioactive compounds was detected under conditions of limited water availability and water stress, as opposed to full water application. This observation underscores the potential for cultivating bush tea in regions characterized by water scarcity without encountering constraints related to nutrient availability.

Another objective pursued in this study was the comprehensive assessment of bush tea's growth, development, productivity, nutritional water productivity, yield, and quality within field conditions, as detailed in **Chapter Four**. The findings presented in this chapter demonstrate the presence of statistically significant distinctions between limited water application as compared to full water application and rainfed conditions. These research outcomes, particularly concerning agronomic management practices, hold considerable potential for the introduction of novel crops into the prevailing agricultural system, thereby contributing to enhanced livelihoods. This initiative aims to address food insecurity and hunger challenges while simultaneously enhancing the yield attributes and quality aspects of bush tea production, thus augmenting the overall value of food production in South Africa. Furthermore, the results unveiled a positive response of various identified compounds to limited water conditions and stress, exhibiting diverse mass-to-charge characteristics. Among these compounds are chlorogenic acids, flavonols, and terpenoids, all of which are present in plants and possess medicinal applications. These findings suggest that the utilization of these compounds has the potential to reduce malnutrition and mitigate food insecurity within the livelihoods of poor rural communities.

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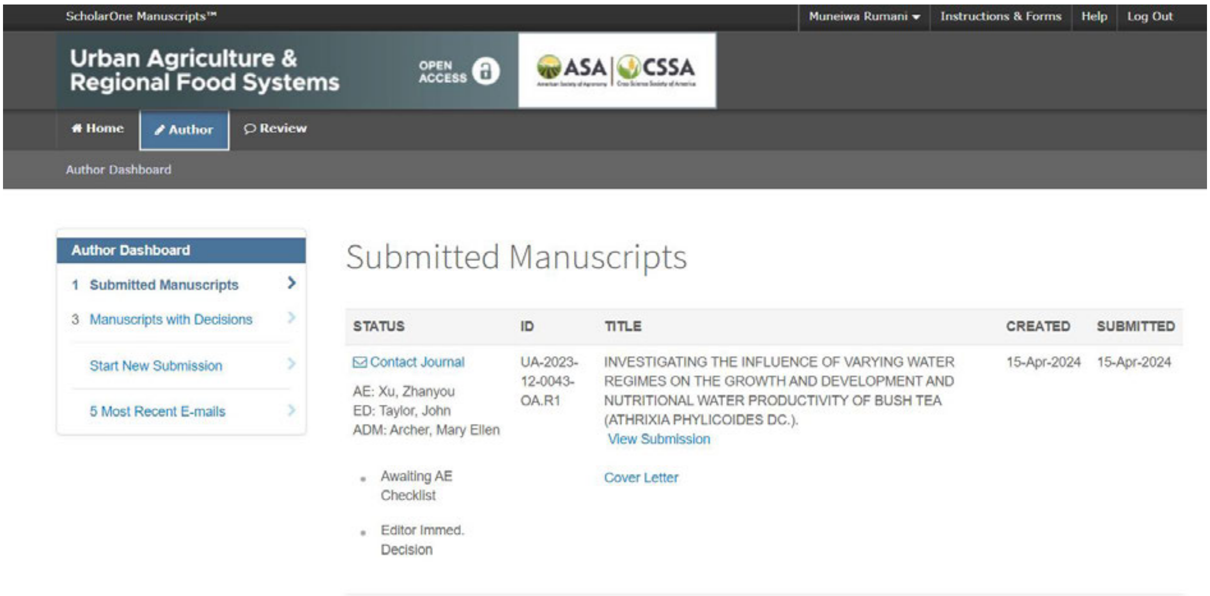
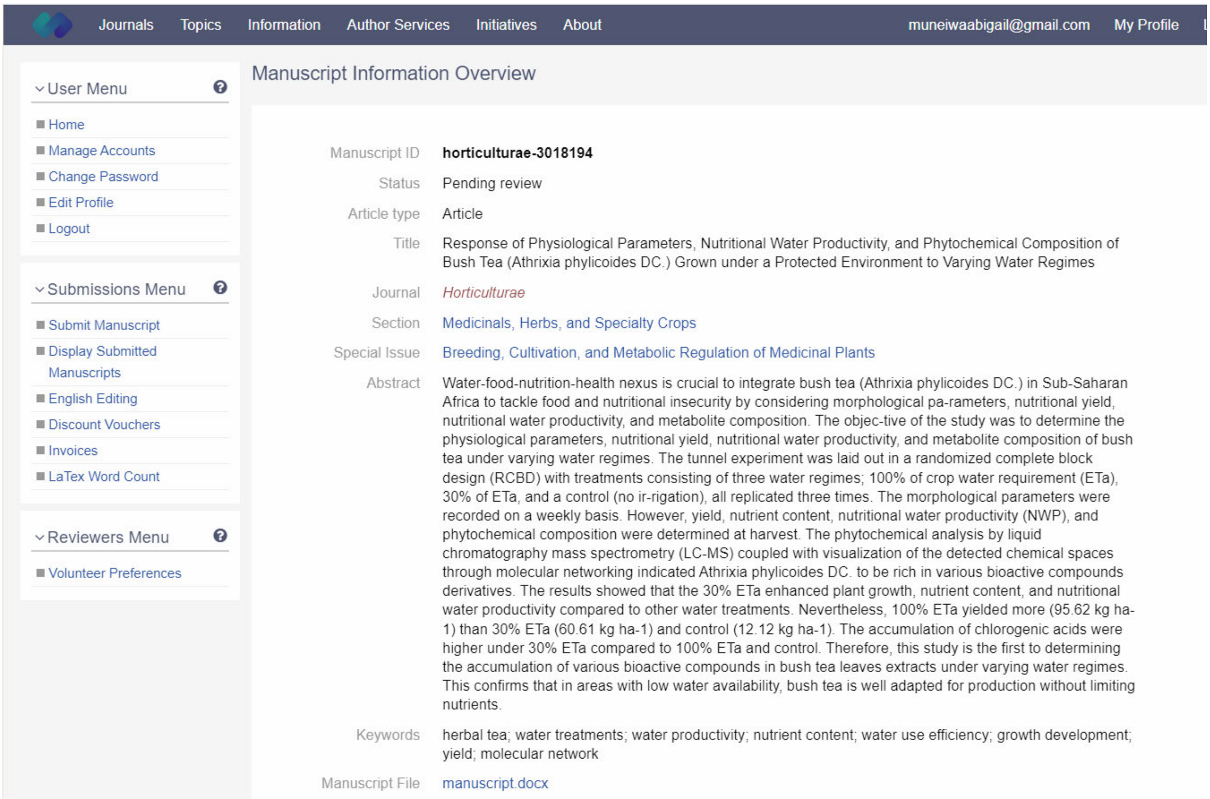
APPENDICES

Submitted manuscript.



Submitted Manuscripts

STATUS	ID	TITLE	CREATED	SUBMITTED
Contact Journal AE: Xu, Zhanyou ED: Taylor, John ADM: Archer, Mary Ellen • Awaiting AE Checklist • Editor Immed. Decision	UA-2023-12-0043-OA.R1	INVESTIGATING THE INFLUENCE OF VARYING WATER REGIMES ON THE GROWTH AND DEVELOPMENT AND NUTRITIONAL WATER PRODUCTIVITY OF BUSH TEA (ATHRIXIA PHYLICOIDES DC.). View Submission Cover Letter	15-Apr-2024	15-Apr-2024



Manuscript Information Overview

Manuscript ID	**horticulturae-3018194**
Status	Pending review
Article type	Article
Title	Response of Physiological Parameters, Nutritional Water Productivity, and Phytochemical Composition of Bush Tea (Athrixia phylicoides DC.) Grown under a Protected Environment to Varying Water Regimes
Journal	*Horticulturae*
Section	Medicinals, Herbs, and Specialty Crops
Special Issue	Breeding, Cultivation, and Metabolic Regulation of Medicinal Plants
Abstract	Water-food-nutrition-health nexus is crucial to integrate bush tea (Athrixia phylicoides DC.) in Sub-Saharan Africa to tackle food and nutritional insecurity by considering morphological pa-rameters, nutritional yield, nutritional water productivity, and metabolite composition. The objec-tive of the study was to determine the physiological parameters, nutritional yield, nutritional water productivity, and metabolite composition of bush tea under varying water regimes. The tunnel experiment was laid out in a randomized complete block design (RCBD) with treatments consisting of three water regimes; 100% of crop water requirement (ETa), 30% of ETa, and a control (no ir-rigation), all replicated three times. The morphological parameters were recorded on a weekly basis. However, yield, nutrient content, nutritional water productivity (NWP), and phytochemical composition were determined at harvest. The phytochemical analysis by liquid chromatography mass spectrometry (LC-MS) coupled with visualization of the detected chemical spaces through molecular networking indicated Athrixia phylicoides DC. to be rich in various bioactive compounds derivatives. The results showed that the 30% ETa enhanced plant growth, nutrient content, and nutritional water productivity compared to other water treatments. Nevertheless, 100% ETa yielded more (95.62 kg ha-1) than 30% ETa (60.61 kg ha-1) and control (12.12 kg ha-1). The accumulation of chlorogenic acids were higher under 30% ETa compared to 100% ETa and control. Therefore, this study is the first to determining the accumulation of various bioactive compounds in bush tea leaves extracts under varying water regimes. This confirms that in areas with low water availability, bush tea is well adapted for production without limiting nutrients.
Keywords	herbal tea; water treatments; water productivity; nutrient content; water use efficiency; growth development; yield; molecular network
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